

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOLUME XL, 1948

CONSISTING OF I-V+790 PAGES,
INCLUDING FIGURES

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LEE ORAS OVERHOLTS

June 23, 1890–Nov. 10, 1946

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XL

JANUARY-FEBRUARY, 1948

No. 1

LEE ORAS OVERHOLTS

FRANK D. KERN

In the death of Lee Oras Overholts at State College, Pennsylvania, November 10, 1946, mycology has lost one of its most distinguished students. His last illness had kept him away from work only for a week. For the last five years his health was impaired but except for short periods he had kept tenaciously at his work. All his life he was a prodigious worker, not sparing himself in health or in illness. Nights, Sundays, holidays and vacations were occasions for furthering the work so well begun during regular days and hours. He was always extravagant with his energy. His extensive collections of specimens, photographs and notes which he has left us are inadequate consolation for his passing but they are evidence of much rich living even though he died at the untimely age of fifty-six years.

Lee Oras Overholts was born June 23, 1890, in Camden, Ohio. He was first married to Flora May Conarroe who died June 7, 1944, and later he was married to Marie Knauz who survives with his four children: Mrs. Charles Rick, of Davis, California; Benjamin and Robert Overholts and Mrs. Eugene Zierdt, all of State College, Pennsylvania.

He attended Miami University, Oxford, Ohio, graduating in 1912 with the A.B. degree. In college he came under the influence of Professor Bruce Fink, an able and inspiring botanical teacher. His early aptitude for scientific work is evidenced by the fact that Professor Fink recommended him during the spring of his senior

[MYCOLOGIA for November-December (39: 627-787) was issued
January 2, 1948]

year (1912) to carry on Arthur's culture work with the rusts. It was there that I first met him and my belief that here was a man of exceptional promise prompted me to offer him in 1915 a position on the staff of the department of botany of the Pennsylvania State College. He accepted the position and held it the remainder of his life, beginning with the rank of instructor and being steadily promoted until he reached a full professorship in 1925.

Three years (1912-15) were spent in graduate work at Washington University (St. Louis) where he held the Rufus J. Lackland Fellowship in the Henry Shaw School of Botany. As an undergraduate he had developed an interest in the "pore fungi." With the aid and encouragement of such men as Fink, Murrill, Bresadola, and C. G. Lloyd he wrote and published his first paper entitled "The Known Polyporaceae of Ohio" at the end of his junior year (June, 1911). This is further evidence of his early scientific maturity. His ability for taxonomic work with the fungi and his interest in the polypores were guided and heightened by association during the graduate years with Dr. E. A. Burt, of the Missouri Botanical Garden. Three important and extensive papers on the Polyporaceae followed in 1914 and 1915 and thoroughly established him as a specialist in that group. Although he worked with all fungi and had an amazing knowledge of all groups, he retained his love for the polypores to the last. He left a voluminous manuscript for a manual of the pileate Polyporaceae of North America north of Mexico practically ready for publication. This represents a compelling interest over a 35-year period of activity.

His Ph.D. degree was conferred by Washington University in 1915. Coming to Penn State that year he first taught general botany and later took over the courses in mycology and tree diseases. He was an excellent teacher. In 1936 he was a joint-author of the HILL, OVERHOLTS and POPP, *Botany, a textbook for colleges*. His knowledge of the fungi especially fitted him for development of the courses in forest pathology. He prepared his own manual for that work and brought together abundant illustrative materials. His graduate courses were always popular and his students came to share his enthusiasm.

Overholts was a great collector. He had a passion for field work and a remarkable ability for inspiring those who accompanied him. He knew where to go not only in the vicinity where he lived but was familiar with many distant localities. Many members of the Mycological Society of America know how much he contributed to the success of the forays which he attended. He was a great believer in photographic records of his fungi. He did his own photography and it could not have been done better. The carefully indexed file of photographs which he made is amazing both for its quality and its magnitude. Looking over the photographic file and then turning to tome after tome of notes and drawings one wonders how time was found for such comprehensive achievements. In addition there were cultures which demanded much time and attention for preparation, care and study. However there was time for all these achievements and also time for recreation for until his health failed him he was an ardent trout fisherman and he always looked forward to the opening of the hunting season.

In the Mycological Society of America, Dr. Overholts served as vice president in 1937, president in 1938, and councilor in 1939. He was a member of Sigma Xi; the Pennsylvania Academy of Science; the Torrey Botanical Club; Phi Beta Kappa; Alpha Zeta; Phi Eta Sigma; Gamma Sigma Pi; and Phi Kappa Tau.

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TAXONOMIC NOTES ON LOUISIANA FUNGI—I

LINDSAY S. OLIVE

(WITH 3 FIGURES)

This paper is one of a proposed series of papers in which the writer plans to present new data on the fungus flora of Louisiana. The description of new species as well as the report of new host records and noteworthy information on distribution of the fungi will be included. Both saprophytic and parasitic species will be considered. The present paper includes a description of several new genera and species of parasitic fungi found in Louisiana, in addition to new distributional and host records for several other species.

PLASMOPARA GONOLOBI (Lagerh.) Swingle

This downy mildew was collected on a species of *Gonolobus* (*Vincetoxicum*). Most of the diseased leaves were also rather heavily covered with the dark brown telia of *Puccinia obliqua* B. & C. The mildew appeared in conspicuous white fuzzy coalescing patches on the under surface of the leaves. Considerable defoliation resulted.

Collected on *Gonolobus* sp.; Baton Rouge, Louisiana; October 23, 1946.

PERONOSPORA PARASITICA (Pers.) Tul.

This mildew, which agrees well morphologically with the descriptions of *P. parasitica*, was found parasitizing an unreported host, *Cleome spinosa* L. This seems to be the first report of the mildew on a member of the Capparidaceae, a family related to the Cruciferae. A downy growth of conidiophores was observed on the under side of the lower leaves. The sporangia (conidia) measured $16-20.4 \times 20.4-27 \mu$. Iwata (1937) refers to a *Peronospora*

sp. on *Cleome spinosa*, but we have no other information on it than this. He apparently did not identify the species of mildew.

Collected on leaves of *Cleome spinosa* L.; Shreveport, Louisiana; December 23, 1946; W. J. Dickson.

PERONOSPORA POTENTILLAE deBary (FIG. 3, A)

This mildew was found on the leaves of dewberry, almost entirely on those leaves already attacked by a rust, *Kunkelia nitens*. The mildew appears as a fuzzy grayish or slightly violaceous growth on the leaves, where it generally produces irregular brown spots of various sizes. Sometimes entire leaves or young shoots are killed by the rust-mildew combination.

The sporangiophores are 5-7 times dichotomously branched, 7.8-11.3 μ in diameter at the base, 535-695 μ in overall height, their stalks 340-575 μ long, and the ultimate branches pointed, recurved, and 5-16.5 μ long. The sporangia (conidia) are oval to ellipsoidal, with pale sordid-violaceous walls, measure 14-18 \times 19-29 μ , and germinate with germ tubes.

Wilson (1914) recognized three species of *Peronospora* on rosaceous hosts in America. These species were listed as *P. potentillae* deBary, *P. rubi* Rabh., and *P. fragariae* Roze & Cornu. He considered *P. rubi* to be the only species of mildew on *Rubus* in this country. Davis (1914, 1919, 1924) reported *P. rubi* on several species of *Rubus* in Wisconsin. The mildew seems to be the same as ours. Fischer (1892) considered all three of the above mentioned species of *Peronospora* synonymous and included them all under the name *P. potentillae*, this name antedating the others. According to him, the only other valid species on members of the Rosaceae is *P. sparsa*.

I am more inclined to agree with Fischer in considering *P. rubi* synonymous with *P. potentillae*. Our fungus actually fits better into deBary's original description of the latter than it does into Rabenhorst's description of *P. rubi*. Unless experimental results prove otherwise, I believe that all these collections of downy mildew on *Rubus* should be classified as *P. potentillae* deBary, of which *P. rubi* is probably best considered a synonym.

Parasitic on leaves of *Rubus* (probably *R. trivialis*); Baton Rouge, Louisiana; April 11, 1947.

MICROSPHAERA ALNI var. *cinnamomi* var. nov. (FIG. 1: 11-15)

Peritheciis amphigenis, disseminatis, 77-142 μ in diametro; parietibus cellularum 10.4-21 μ in diametro, ascos 2-8 continentibus; appendicibus 1.5-2 \times diametrum peritheciis, 137-306 μ longis, 1-6 \times dichotome ramosis (raro haud ramosis), apicibus ultimis non recurvis. Ascis 27.8-46.1 \times 47-67 μ , 2-7 sporogenis; ascosporis 10.5-16.5 \times 18.3-25.5 μ . Parasitica in plantulis juvenilibus *Camphorae cinnamomi* Nees & Eberm.

Perithecia scattered over upper and lower surfaces of the leaves, 77-142 μ in diameter, wall cells 10.4-21 μ in diameter, containing 2-8 asci; appendages 1.5-2 times the diameter of the perithecia, measuring 137-306 μ in length, 1-6 times dichotomously branched, rarely unbranched, the ultimate tips not recurved. Asci short-stalked, measuring 27.8-46.1 \times 47-67 μ , 2-7 spored; ascospores 10.5-16.5 \times 18.3-25.5 μ .

Parasitizing young seedlings of *Camphora cinnamomi* Nees & Eberm.; Baton Rouge, Louisiana; February, 1947; Q. S. Holde-
man.

The diseased seedlings were found growing beneath the parent tree. The mildew produced grayish white spots on both leaf surfaces, or often covered almost the entire leaf surface. Conidia were not being produced, but a few old conidia, typical in form, were observed under the microscope.

Salmon (1900), in his monograph of the Erysiphaceae, lists several varieties of *M. alni* and points out that the species is probably the most variable in the Erysiphaceae. Apparently, the most important single consideration in distinguishing the different species and varieties of *Microsphaera* is the character of the appendages, and even the appendages often show considerable variation within the species or variety.

The present fungus differs from typical *M. alni* and its known varieties in the more variable mode of branching of its appendages, in the failure of the ultimate branches to show any recurving, and in its parasitism of camphor seedlings. The present variety approaches the description of *M. alni* var. *lonicerae* (DC.) Salm., the appendages of which generally show no recurving of the ter-

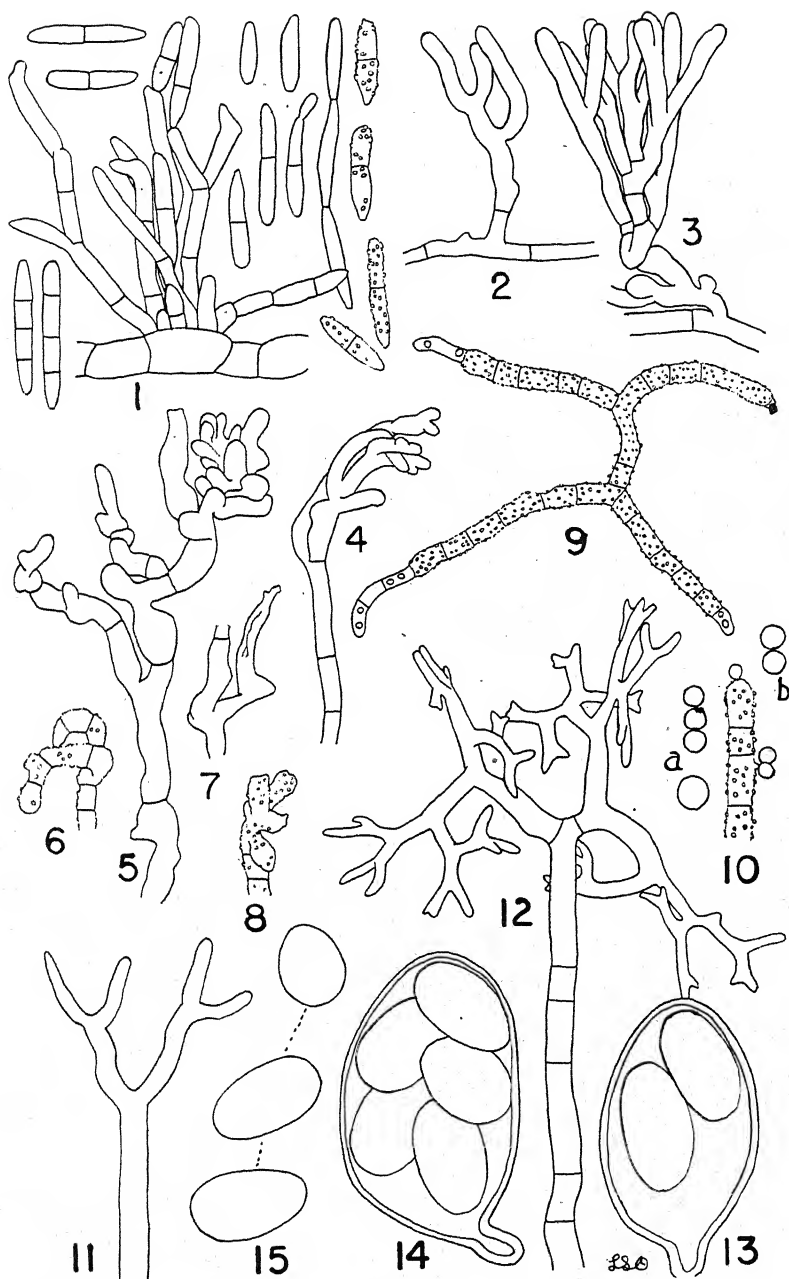


FIG. 1. 1, *Ramularia sidae*; 2-10, *Thallospora aspera*; 11-15, *Microsphaera alni* var. *cinnamomi*.

minal branches, but the latter is parasitic on species of *Lonicera* and has appendages only 3-4 times dichotomously branched. The two differ in a few other details of minor importance. It would be interesting to know whether these varieties of *M. alni* are capable of growing on the hosts of typical *M. alni* such as alder and oak and, if so, whether they would continue to exhibit what we are considering varietal differences, or whether they would revert to the typical form. Experimental evidence is needed here.

BUBAKIA CROTONIS (Cke.) Arth.

This interesting rust was found on the leaves of *Croton punctatus*. Uredinia and telia occurred on both sides of the leaves on small brown leaf spots. The collection was made at Grand Isle, Louisiana; November 30, 1946.

PHYSOPELLA FICI (Cast.) Arth.

This rust is common in our area on leaves of *Ficus carica* and appears to be the cause of considerable defoliation of that plant. The uredinial phase is abundant on the leaves, but we have not found telia. We also have a collection of this rust (uredinia) on *Morus rubra*. Although there are earlier reports of it on *Morus* in other countries, we have found no previous records of its occurrence on this particular species of mulberry. The uredinia in this collection are brown, amphigenous, but more abundant on the lower surface, with paraphyses around the periphery, and with uredospores that measure $14.4-22.5 \times 20.6-34 \mu$. The collections were made at Baton Rouge, during the fall months of 1946.

USTILAGO SPHAEROGENA Burrill

This smut, affecting the heads of a grass, *Echinochloa colonum*, produced spores that measured $6-7 \times 7.1-8.7 \mu$. Diseased grains are somewhat enlarged, but the smut is not conspicuous until the grain is crushed. The collection was made by Dr. Clair A. Brown at Thibodaux, Louisiana; October, 1946.

Ramularia sidae sp. nov. (FIG. 1:1)

Hypophyllis, areolas pulveraceas et albas proferentibus, 1-5 mm. in diametro; conidiophoris racematis per stomata perrumpentibus, simplicibus vel ramosis, septatis; conidiis a termino latereque gestis, saepe catenulatis, cellulas 1-4 habentibus, $2.3-4.6 \times 10.4-40 \mu$, maturis parietis asperos frequenter gerentibus. Parasitica in foliis *Sidae* sp.

Forming powdery white patches on the under surface of the leaves, measuring 1-5 mm. in diameter; leaf tissue generally not discolored, or only slightly yellowish in spots where the fungus is sporulating; symptoms generally not apparent on the upper leaf surface. Conidiophores arising in clusters through the stomates, simple or branched, septate, producing conidia terminally and laterally, conidia frequently catenulate, 1-4-celled, measuring $2.3-4.6 \times 10.4-40 \mu$, often with small scattered warts over the surface at maturity.

On leaves of *Sida* sp.; Baton Rouge, Louisiana; January 13, 1947.

This seems to be the only species of *Ramularia* described on *Sida*, and it is apparently a distinct new species. A specimen of *Ramularia* on an unidentified species of *Sida*, collected by Shear at Winter Park, Florida, was recently forwarded to me by Mr. John A. Stevenson. The fungus proved to be identical with ours.

Thallospora gen. nov.

Parasitus systemicus, sine conidiophoris bene delineatis, conidiis ex hyphis ramosis excrescentibus, gracilibus, dichotome ramosis, multiseptatis, in massa alba intra hospitis ovarium factis.

Systemic parasite; no well-developed conidiophores present, conidia developing as direct outgrowths from branching hyphae, slender, dichotomously branched, multiseptate, hyaline, produced in a white mass inside the ovary of the host.

The type species is *T. aspera*.

The genus is placed in the family Moniliaceae of the Moniliales. There has been some doubt as to what tribe of that family would best accommodate the genus. The Staurosporae, as defined by Clements and Shear, would appear to be the most satisfactory. According to these authors, the tribe is characterized by "conidia forked or lobed, radiate or stellate, hyaline or bright colored, septate or continuous." The conidia of our new fungus are usually

several times dichotomously forked, the branches typically spreading out in radiate fashion.

***Thallospora aspera* sp. nov. (FIGS. 1:2-10; 3, B-E)**

Parasitus systemicus et obligatus. Hyphae per maximum partem hospitibus penetrantibus, in ovario sporogenis; conidiis in massa alba in ovario effectis, gracilibus, dichotome ramosis, multiseptatis, parietes asperos habentibus, paulum hygroscopicibus, 68-237 μ longis, ramis 4.5-9.5 μ in diametro; germinantibus ut tubulos germinum breves et septatos efficiant, vel microconidia globosa ex ulla cellula gerentibus, microconidiis 2.5-5.2 (6.5) μ in diametro. Parasitica in *Veronica peregrina* L.

A systemic and obligate parasite, with hyphae penetrating throughout most of the host, sporulating in the ovaries of the host; conidia produced as direct outgrowths of branching hyphae, breaking away at maturity and filling the capsule, several times dichotomously branched, multiseptate, rough-walled, somewhat hygroscopic, measuring 68-237 μ in length, the branches 4.5-9.5 μ in diameter, germinating to produce short septate germ tubes from the ends of the branches, or budding out small globose conidia terminally and laterally, microconidia measuring 2.5-5.2 (6.5) μ in diameter.

Parasitizing *Veronica peregrina* L.; Baton Rouge, Louisiana; March-April, 1947.

The fungus was just called to the writer's attention by Dr. C. W. Edgerton, who had observed it for a number of years around Baton Rouge. Infected plants in the field are not distinguishable from diseased plants until the capsules open. Then it can be seen that the capsules of infected plants are filled with white masses of conidia in which tiny brown, aborted seeds are imbedded. Generally all capsules of an infected plant become filled with conidia. Free-hand sections through various parts of these plants show that the hyphae may be present anywhere except in the smaller roots. They appear in the upper part of the primary root, in the stem up to the top of the plant, frequently in the leaves, and in all parts of the flower. Hyphae pass from the stem through the peduncle of the flower and then into the petals, ovary, style, stigma, and through the filaments of the stamens into the anthers. In the anthers, some of the hyphae wind about among the pollen grains and apparently destroy a number of them.

The organism sporulates only in the ovary. Here the hyphae penetrate mainly through the septum and placentae out into locules. Some enter the funiculi of the ovules. These developmental phases may all be observed in young flower buds several days before they open. Up to this stage of development all hyphae have been intercellular. No haustoria are produced by the fungus. Soon after hyphae penetrate the funiculus, a few of them continue on up into the tissue of the ovule itself, mostly in the nucellar region at first. Later, when the seeds are approaching maturity, the hyphae within them are observed to be much more abundant. Some penetrate the endosperm, while others may enter the embryo. At this time, some of the hyphae within the seed are seen to be intracellular, narrowing considerably where they pass through the cell walls. By the time the capsule opens, the seeds all appear brown, hard, and somewhat shrunken. None of them seems to be capable of germinating. However, it is possible that these aborted seeds, which fall to the ground, may serve as a means by which the fungus is carried over in the soil until the new infections take place.

During these developments within the ovules and seeds, the hyphae which have passed out into the locules repeatedly fork in dichotomous fashion to produce fascicles of branches (FIG. 1: 2-4). At maturity the fascicles break up into their component parts, the conidia. The conidia are hyaline, rough-walled, multiseptate, and dichotomously branched. According to Vuillemin's definition, such conidia, which are actually a part of the hyphae bearing them and not distinct structures formed *de novo*, are termed *thallospores*. Remains of the hyphae which give rise to the conidia may be found in the ovary after the conidia are shed (FIG. 1: 5-8).

When placed in water the conidia show a hygroscopic tendency to spread out in radiate fashion (FIG. 1: C, D). On nutrient agar, they may germinate terminally to produce short septate germ tubes, which cease developing early (FIG. 1: 9). In water and on some agars numerous spherical microconidia are budded out from any cell (FIGS. 1: 10; 3, E). They measure 2.5-5.2 (6.5) μ in diameter. On agar some of the cells of the macroconidia swell into chlamydospore like structures (FIG. 3: E). All attempts to grow the fungus in culture have failed.

I have studied some slides of the fungus prepared by Dr. M. T. Cook with the iron alum haematoxylin technique. Sections through diseased ovaries of the *Veronica* revealed the presence of one to several nuclei (mostly 2-4) in each hyphal cell, and one or two nuclei in each cell of the macroconidia.

Greene (1943) reports finding this same fungus on *Veronica peregrina* in Wisconsin, but he makes no attempt to classify or formally describe it. He thought that the fungus occurred only in the fruits and seeds. He was unable to find hyphae passing through the flower peduncles.

***Ormathodium ambrosiae* sp. nov. (FIG. 2: 1-11)**

Maculis hypophyllis; 1-5 mm. in diametro; fungo in forma incrementi plumosi, purpureobrunnei se ostendenti; hyphis in folio numerosis, inter cellulas sitis; conidiophoris per stomata emergentibus, subbrunneis, aliquando roseis, multiseptatis, semi-erectis, super pilos folii saepe ascendentibus atque ab apicibus eiusdem se diffudentibus. Conidiis a termino latereque singulis aut duobus tribusve catenatis, in colorem subbrunneum se vertentibus, 1-5 septatis, $3.8-7.3 \times 22-68 \mu$, a termino vel latere germinantibus. Parasitica in foliis *Ambrosiae trifidae* L.

Spots chiefly hypophyllous, 1-5 mm. in diameter, fungus appearing as a fuzzy purplish brown growth; hyphae abundant in the leaf, intercellular; conidiophores emerging through the stomates, chiefly on the under surface of the leaf, brownish, sometimes rose-tinted, multiseptate, semi-erect, often climbing up the leaf hairs and spreading out from their tips. Conidia borne terminally and laterally, singly or in chains of 2 or 3, becoming brownish, 1-5-septate, $3.8-7.3 \times 22-68 \mu$, germinating terminally or laterally.

Parasitic on leaves of *Ambrosia trifida* L.; Baton Rouge, Louisiana; September-October, 1946.

The fungus was obtained in culture on nutrient agar, but grew very slowly in the form of whitish or almost cream-colored yeast-like colonies. The colonies were composed chiefly of slender filiform conidia produced in great abundance on the hyphae. Inoculations were not attempted.

Sydow (1928) founded the genus *Ormathodium* to accommodate a parasite, *O. styracis*, on *Styrax argenteum*. It seems to be similar in several ways to our fungus on ragweed. According to Sydow, *O. styracis* has olive brown, 1-2-septate conidia in simple

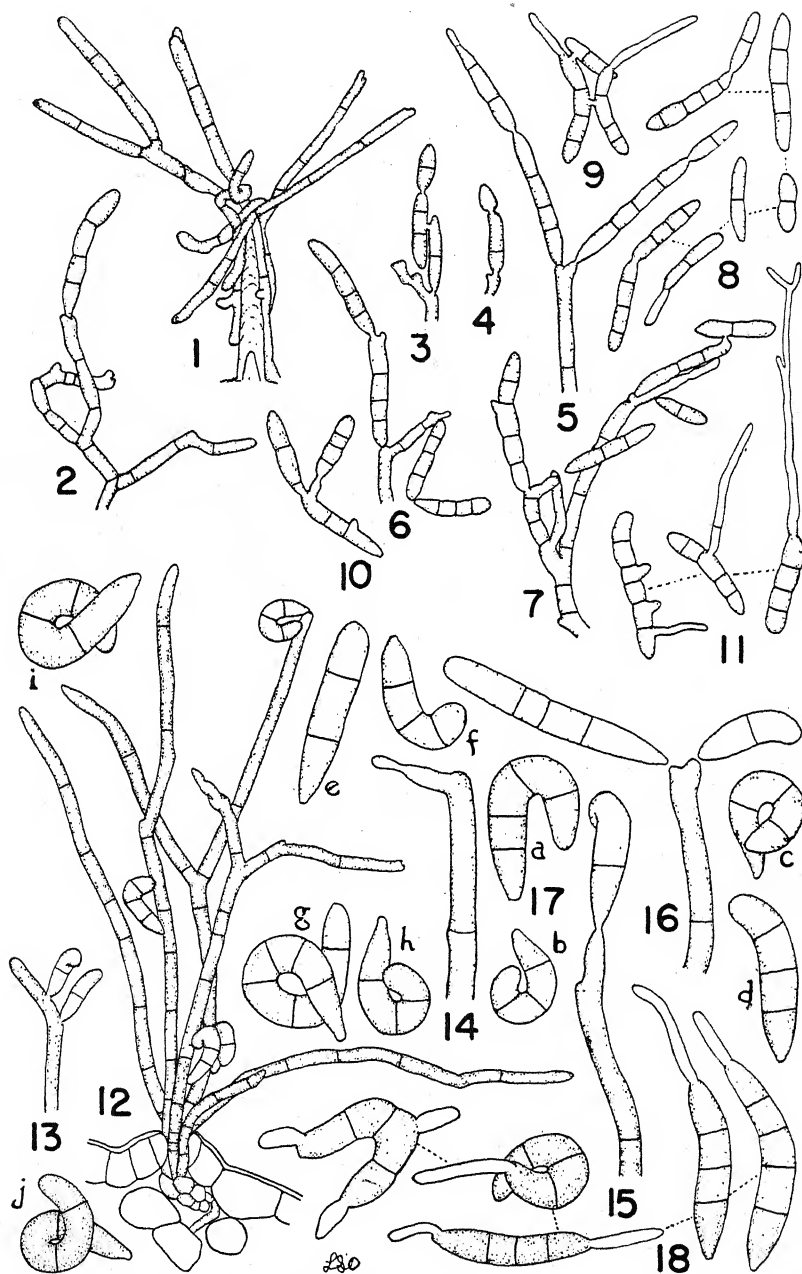


FIG. 2. 1-11, *Ornathodium ambrosiae*; 12-18, *Helicomina caperoniae*.

or branched chains, and its conidiophores also tend to spread out from the tips of the leaf hairs.

Clements and Shear (1931) reduce the genus *Ormathodium* Syd. to synonymy with *Dendryphium* Wallr. However, *Dendryphium* was founded on a saprophytic species (*D. comosum* Wallr.), and practically all species placed in the genus since then have been saprophytes. Even some of these saprophytes appear to have been incorrectly put into this genus. *Ormathodium* differs from *Dendryphium* in at least one very important feature, its distinctly parasitic nature. This would appear sufficient reason for considering *Ormathodium* a valid genus. To place parasitic forms in *Dendryphium* would serve only to increase the confusion already existing within that genus.

At least one other previously described species placed in *Dendryphium* should be transferred to *Ormathodium*. This is *D. costaricense* Spegaz. It was described by Spegazzini (1919) as a parasite on leaves of *Xylosma Salzmanni* Eichl. in Central America. Saccardo (1931) has already questioned its position in *Dendryphium*, pointing out that its parasitic nature might distinguish it as the type for a new genus. Saccardo appears to have overlooked Sydow's genus, *Ormathodium*.

I propose the following new combination for Spegazzini's fungus: ***Ormathodium costaricense* (Spegaz.) comb. nov.**

***Helicomina* gen. nov.**

Parasitica in plantis generosis; conidiophoris elongatis, fuscis, simplicibus vel ramosis, multiseptatis; conidiis a termino latereque partis, rectis, curvatis, glomeratis, pro longitudine validis, haud hydropiscis, septatis, coloratis. Coloniae in formis fuscarum areolarum plumosarum praecipue se ostendunt.

Parasitic on higher plants; conidiophores elongate, dark, simple or branched, multiseptate; conidia produced laterally and terminally, straight, curved, or coiled, stout in proportion to the length, not hygroscopic, septate, colored. Colonies appearing as dark fuzzy patches or sometimes effuse. Except for its parasitic nature and the presence of straight as well as coiled conidia, the genus resembles *Helicoma*.

The type species is *H. caperoniae*.

Helicomina caperoniae sp. nov. (FIGS. 2: 12-18; 3: F, G)

Praecipue hypophyllis, maculas fuliginosas, 4-10 μ in diametro, efficientibus vel effusis; mycelio inter cellulas sito; conidiophoris per stomata racematis, simplicibus vel subramosis, brunneis, 2-12-septatis, 4-6.6 \times 92-329 μ , conidia et a termino et a latere ferentibus; conidiis rectis, curvis, vel glomeratis, 1-7-septatis, plerumque triseptatis, pallido-brunneis, hilum parvum habentibus, 4.5-6.3 \times 17.4-40 μ , spiris 12.2-19.1 μ in diametro, terminibus vel latere germinantibus. Parasitica in foliis *Caperonia castaneaefoliae* (L.) St. Hil.

Chiefly hypophyllous, producing sooty spots 4-10 mm. in diameter, or becoming effuse; internal mycelium intercellular, without haustoria; conidiophores appearing in clusters of several through the stomates, simple or few-branched, brown, mostly 2-12-septate, measuring 4-6.6 \times 92-329 μ , bearing the conidia both terminally and laterally; conidia straight, curved, or up to $1\frac{1}{3}$ times coiled, 1-7-septate, mostly 3-septate, pallid brown, with a small hilum at the proximal end, the straight conidia measuring 4.5-6.3 \times 17.4-40 μ , the coils (measured at right angles to the long axis of the conidiophore) 12.2-19.1 μ in diameter, germinating at both ends and sometimes laterally.

Parasitic on leaves of *Caperonia castaneaefolia* (L.) St. Hil.; Baton Rouge, Louisiana; October 2, 1946; Q. L. Holdeman.

The genus *Helicomina*, as defined here, is distinguished from *Helicoma* chiefly on the basis of its distinctly parasitic nature. The genus *Helicoma*, as defined by Linder (1929), is made up of saprophytic species. Our fungus further differs from all known species of *Helicoma* in its tendency to produce a large number of straight conidia in addition to the curved or coiled ones. This latter character, however, should probably not be considered of much generic value.

In his key to the genera of the Helicosporous Fungi Imperfecti (1929) and in a later paper (1931), Linder distinguishes the genus *Helicoceras* (*Gyroceras*) from the other genera mainly on the basis of its parasitism of vascular plants and in its possession of toruloid spores and reduced conidiophores. Our fungus differs from *Helicoceras* in the possession of well-developed conidiophores and in the failure of the conidia to show any conspicuous constrictions in the region of the septa. Although the conidia of *Helicomina caperoniae* produce short germ tubes when they are placed in water or on nutrient media, they cease developing early. Attempts to grow the fungus in artificial culture have failed.

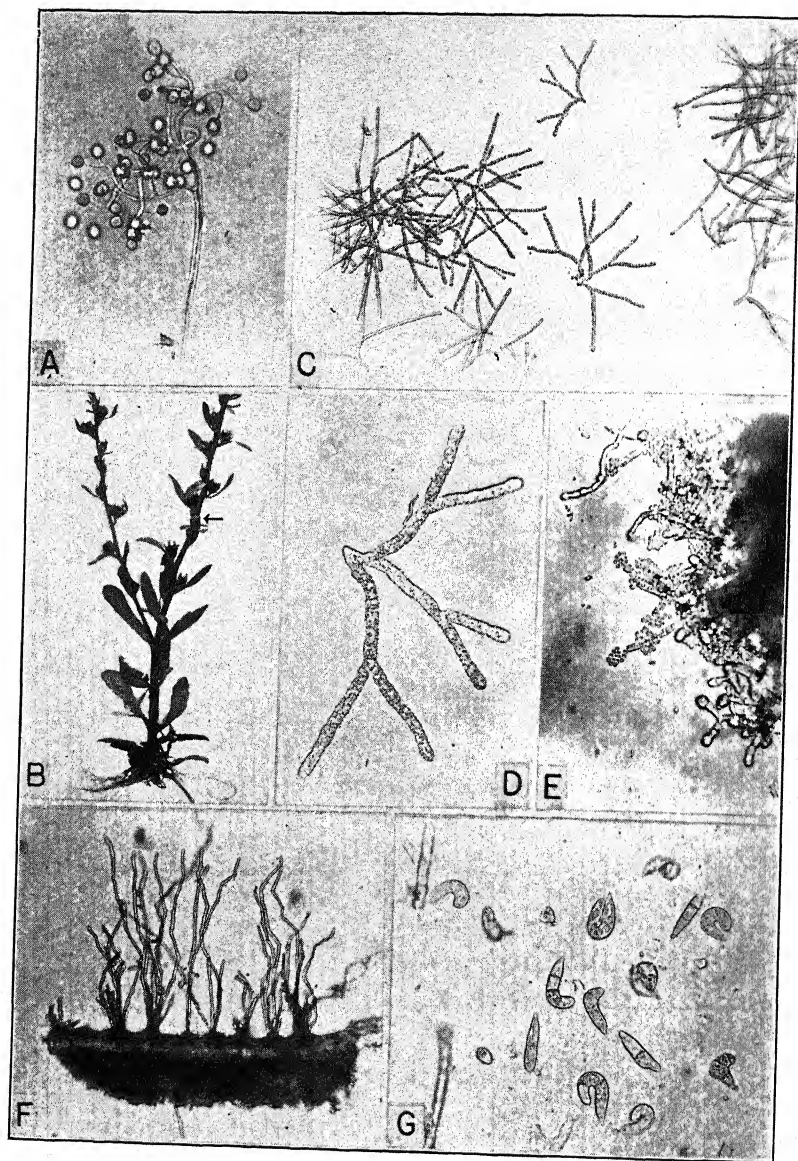


FIG. 3. A, *Peronospora potentillae*; B-E, *Thallospora aspera*; F, G, *Helicominia caperoniae*.

The writer is grateful to Dr. C. W. Edgerton for making the photographs, to Dr. P. G. Moorhead of this university for preparation of the Latin descriptions, and to Mr. John A. Stevenson of the Beltsville Plant Industry Station for information on host records.

Type specimens of new species of fungi described in this paper have been deposited in the herbarium at Louisiana State University and in the mycological herbarium at the Plant Industry Station of the United States Department of Agriculture, Beltsville, Maryland.

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EXPLANATION OF FIGURES

FIG. 1. *Ramularia sidae*, 1. Conidiophores and conidia. *Thallospora aspera* (2-10). 2-4, dichotomously branched hyphae developing into conidia; 5-8, hyphae after conidia have broken away; 9, conidium germinating; 10, portion of a conidial branch producing microconidia (*a, b*). *Microsphaera alni* var. *cinnamomi* (11-15). 11, 12, perithecial appendages; 13, 14, asci; 15, ascospores. (All drawings $\times 550$, except 9, $\times 355$, and 10, $\times 830$.)

FIG. 2. *Ormathodium ambrosiae* (1-11). 1, Conidiophores diverging from the tip of a leaf hair; 2, branched conidiophore with conidium; 3, conidia fusing laterally while on conidiophore; 4, young conidium being produced apically from an older one; 5, 6, conidiophores with conidia in chains; 7, conidiophore with conidia developing at various points; 8-11, conidia, some showing anastomoses and some germinating. *Helicomina caperoniae* (12-18). 12, tuft of conidiophores arising through stoma of leaf; 13, conidiophore producing two conidia laterally; 14-16, conidiophores producing conidia terminally; 17, conidia of various shapes (a-j); 18, conidia germinating. (Nos. 1-13, $\times 360$; nos. 14-18, $\times 650$.)

FIG. 3. *Peronospora potentillae*. A, sporangiophore. *Thallospora aspera* (B-E). B, diseased plant, showing white mass of conidia in the seed capsules (arrow); C, group of mature conidia; D, single enlarged conidium; E, conidia on agar, showing the production of microconidia and chlamydo-spore-like swellings. *Helicomina caperoniae* (F, G). F, transverse section of leaf showing clusters of conidiophores arising through the stomates; G, conidia.

TELIOPORE DISCHARGE IN PUCCINIA TUMIDIPEs PECK¹

STUART M. PADY²

(WITH 19 FIGURES)

In the Uredinales the discharge of teliospores has not been reported as far as the writer has been able to determine. The occurrence of discharged teliospores of *Puccinia tumidipes* Peck on the leaves of *Lycium halimifolium* (Matrimony Vine) in the vicinity of the telial sorus is therefore of interest.

Puccinia tumidipes is known only in the uredial and telial stages although Arthur³ has described the pycnial and aecial stages on the basis of a single culture made from teliospores on *L. pallidum* sown on *L. halimifolium*.⁴ From two sowings, a few pycnia were produced in each case and in one a single uredial sorus was formed. The rust was therefore considered to be autoecious although no field collections of the aecial stage have ever been made. On the campus of Kansas State College there is a large clump of the host which has been there for many years and which is rusted year after year. Uredia and telia appear early in the growing season and the heavily infected leaves tend to become yellow and to be cast prematurely, particularly when the weather turns hot and dry. Usually there is a second period of infection late in the growing season. Other bushes on the campus and in the town are free from rust suggesting that in this long-established clump of bushes the rust is able to overwinter, presumably in the uredial stage.

¹ Contribution No. 488, Department of Botany and Plant Pathology, Kansas State College, Manhattan, Kansas.

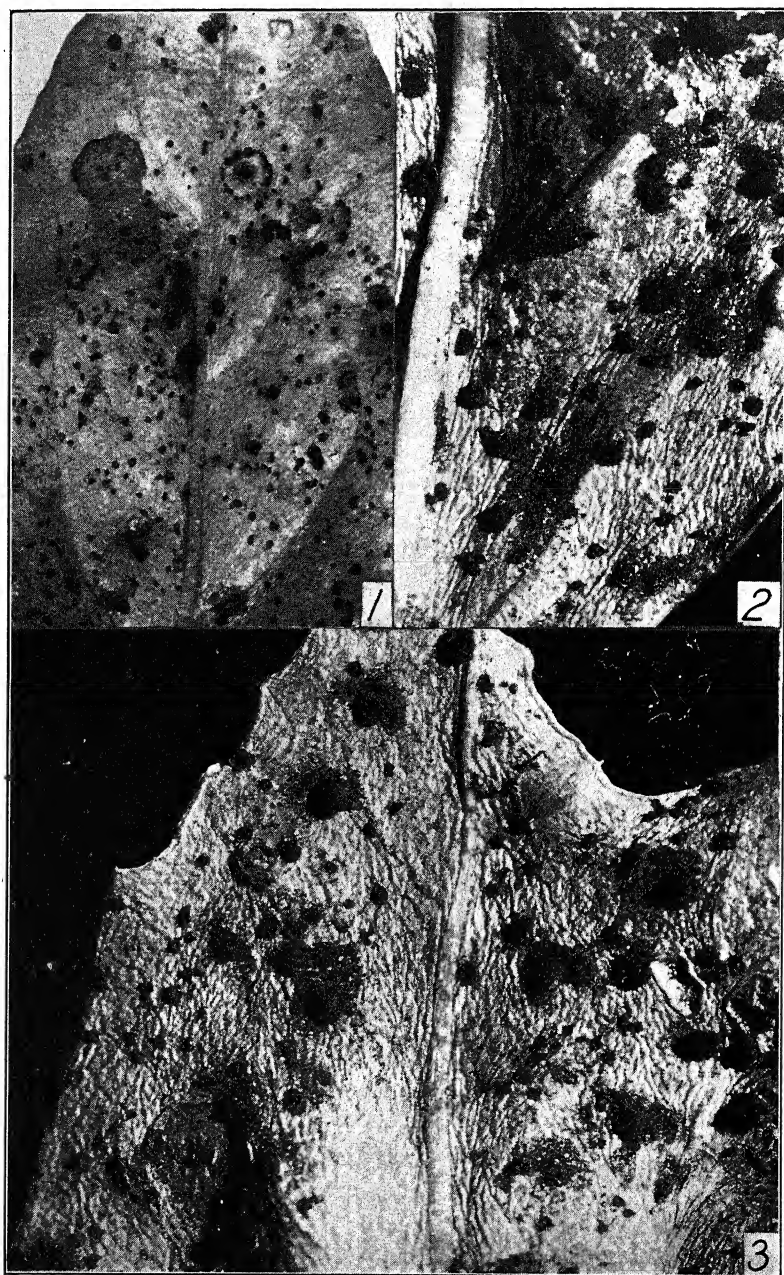
² This work was done while the author was Assistant Professor in the Department of Botany and Plant Pathology, Kansas State College.

³ Arthur, J. C. Manual of the rusts of the United States and Canada. Purdue Research Foundation, 1934.

⁴ Arthur, J. C. Cultures of the Uredineae in 1915. *Mycologia* 8: 136. 1916.

On June 14, 1946, primary and secondary uredia were abundant on the lower surface of many of the leaves, with here and there an occasional black telial sorus (FIG. 1). Occasionally the telia were mixed with the uredia but usually they were separate. In the vicinity of some of the telia an unusual darkening of the surface was noted. The discoloration was on one side of the sorus extending fan-wise 1 to 5 mm. (FIGS. 1-3). It was black, granular, fairly uniform and had the mold-like appearance of one of the Dematiaceae of the Moniliales although the leaf tissues below were green and apparently normal. When portions were removed for examination it was discovered that it was firm and crust-like, even semi-elastic, and the removal of a small portion was likely to dislodge the remainder. The constituent particles were so firmly fused to one another that it suggested the drying down of a gelatinous mass. Under the hand lens small black shining bodies could be seen, which under the microscope were shown to be teliospores. Since this suggested that the teliospores had been discharged from the surface a careful examination of the discharged spores was made in an attempt to determine the mechanism involved.

The most striking feature of the discharged teliospores was that some had pedicels whereas others apparently lacked this structure: yet even in these there were indications of the remains of the pedicel in the form of a transparent membranous material surrounding the spore (FIG. 9). This membrane was attached at the basal end of the teliospore but free at the upper end (FIG. 17C), suggesting that the pedicel had actually reversed itself around the spore. This unexpected activity of the pedicel was confirmed by later observations and experiments. The discharged spores were therefore of two types, those with pedicels in normal position, and those whose pedicels surrounded the spore: for convenience the former will be referred to as pedicellate, the latter as reversed. Moreover, the pedicellate spores invariably possessed ruptured pedicels which had been broken near the base. A normal teliospore when removed from a sorus has a pedicel longer than the spore with an upper expanded portion occupying about two-thirds or more of the length and a lower unexpanded portion which is narrow, tapering, and has a roughened surface (FIG. 12). Discharged



FIGS. 1-3. *Puccinia tumidipes*.

spores usually have the pedicel ruptured at the junction of these two regions (FIGS. 13, 14).

Valuable information was obtained by mounting the discharged spores from the dry film on a dry slide, adding a cover-slip and making drawings and measurements *before* water was added. When water was run under the cover-slip two remarkable activities took place: first, the spores in the film moved rapidly apart and were often pushed completely out of the field and secondly, the spores with pedicels snapped downward and the pedicel suddenly turned inside out. In several cases it was possible to make camera lucida drawings of individual dry pedicellate spores (FIGS. 17A, 18A), then drawings of the same spores after the pedicels had reversed (FIGS. 17B, 18B). From many such observations it was concluded that the structure of the pedicel accounted for both of these activities. There is an outer, thin, tough, elastic layer, and an inner layer of highly hygroscopic material which is indicated in the drawings by a dotted line (FIGS. 12-19). When a spore is discharged the pedicel ruptures at the base and under certain conditions turns inside out surrounding the spore. On the leaf both types are found (FIG. 9) but the reversed type usually predominates.

This inner hygroscopic layer can be demonstrated by mounting the pedicellate spores in lactophenol with a trace of cotton blue and warming the slide to drive off the water and stain the spore. When this is done this layer is clearly shown as a spindle-shaped band extending from the top of the pedicel to the ruptured base, becoming progressively narrow toward each end (FIGS. 13, 14).

The existence of this highly hygroscopic layer was also shown by adding water to the dry discharged spores and then replacing the water by India ink (FIG. 10). By this method it was shown that this inner layer enlarges greatly where it is in free contact with the water. In pedicellate spores the exposed area is relatively small, but even here some swelling takes place (FIG. 16). In the reversed spores this inner layer is now external and water is rapidly absorbed so that it swells to many times its thickness (FIGS. 18B, 19). The photomicrograph in figure 10 shows this very clearly. Actual measurements show that the total diameter of the spore and two layers may be 60-65 μ with the individual

layers 15–20 μ with a maximum of 30 μ . In pure water (FIG. 17C) or lactophenol (FIG. 9) this layer would not be detected as it is almost perfectly hyaline and gelatinous but with India ink the boundaries could be accurately determined.

The rapid swelling of this inner gelatinous layer would explain the two visible activities which occur when water is added to the dry discharged spore layer. Since most of discharged spores are of the reversed type this inner layer is now outer, exposing a large surface area capable of rapid water absorption. This rapid swelling of the closely-packed spores in the dry film forces them apart and since each spore may reach an outside diameter including the two gelatinous layers of 65 μ , it means that a tremendous expansion takes place (FIG. 10). In nature the spore mass would be free to swell equally in all directions; on the slide, however, the expansion is restricted and the pedicels enlarge rapidly forming a flat sheet. In the pedicellate spores the rapid expansion of this layer would set up a tremendous pressure inside the pedicel. The tough outer wall is capable of resisting this pressure, but the exposed torn end of the pedicel lacks the outer wall at that point. The swelling gelatinous layer can, if the break is large enough, force itself out of the free end and thus partially relieve the pressure (FIG. 16).

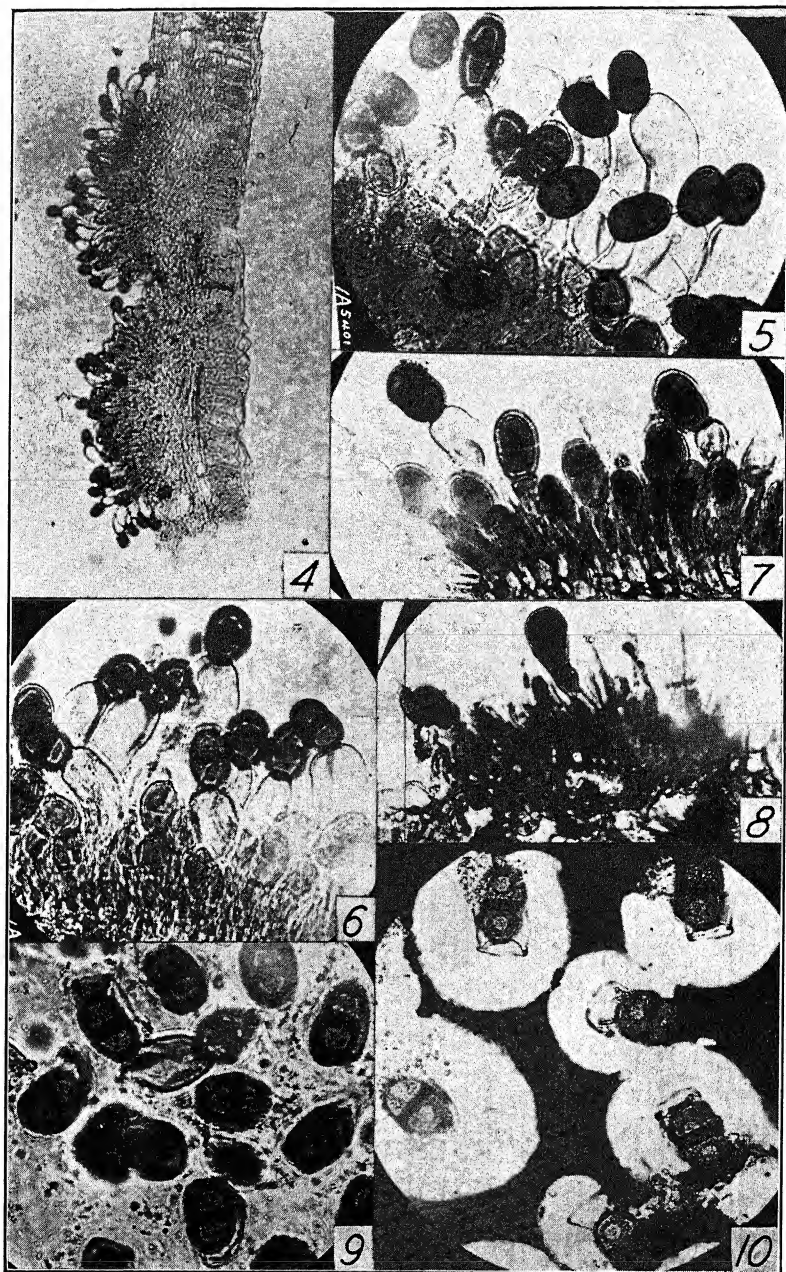
The lower exposed portion of the pedicel would develop a considerable pressure immediately, due to rapid water absorption. The pressure would be much greater than in the upper lining of the pedicel since the upper end is further from the water. Moreover the hygroscopic layer is not continuous across the upper part of the pedicel so that at the point of attachment the pressure would be practically zero. The net result of this rapid increase in pressure is a stretching of the pedicel wall with the pressure highest at the base, gradually less toward the top and zero at the extreme upper end. The lower portion opens out in a funnel-shaped manner and the lower edge begins to turn back (FIG. 15). As soon as the inner wall pressure at the free end is greater than the pressure exerted by the stretched outer wall, the outer wall is doubled backward upon itself. The pressure of the inner wall is building up so rapidly that as soon as the outer wall begins to fold back there is a sudden movement and the pedicel completely re-

verses itself. The inner wall is now on the outside and the pedicel wall stretches over the spore with a fold at the point where the outer wall doubles backward. On the slide, the cover slip and glass slide tend to hold the gelatinous inner layer in place and as a result the teliospore itself is pulled downward.

It should be emphasized that this turning inside out of the pedicel occurs so suddenly that no intermediate stages can be observed. If under low power of the dissecting microscope a discharged pedicellate spore is mounted dry on a slide with cover slip holding the specimen in place, and water is run in under the cover slip, a sudden movement of the spore takes place as soon as the water strikes the spore, and the pedicel disappears. So sudden is this transition that one can hardly believe that it has actually taken place; repeated observations however always give this almost instantaneous reaction.

Sometimes the spore turns inside out with the outer wall forming a very prominent bell where it folds backward (FIGS. 10, 18*B*). The teliospore in figure 17*A* reversed itself when water was added and a prominent bell or funnel-shaped structure appeared (FIG. 17*B*). Then almost as soon as the camera lucida sketch had been made a further shift took place and the teliospore was pulled further down inside the tube formed by the reversed pedicel wall (FIG. 17*C*).

The base of the pedicel sometimes shows an irregular surface of fine lines or ridges suggesting granules of various sizes. This is observable in dry mounts (FIG. 18*A*), in mounts in water (FIGS. 10, 15, 16), and particularly in the reversed spores (FIG. 18*B*). In lactophenol this roughened surface was not evident. The presence of this layer is of diagnostic value in determining whether or not the pedicel actually surrounds the spore, or, as was at first thought, merely lies to one side of it. The spore in figure 18*A* was mounted dry and drawn with camera lucida, water was then added with the result that the pedicel immediately reversed. The water was replaced by India ink and the spore redrawn (FIG. 18*B*); the granulations are now located around the upper part of the teliospore (FIG. 18*B*). These markings are shown clearly in the photomicrograph in figure 10, especially in the spore at the extreme left and also in the upper left hand corner.



FIGS. 4-10. *Puccinia tumidipes*.

The presence of this gelatinous layer on the outside of the reversed discharged spores explains why the spores were held in a firm film. As the conditions for discharge required the presence of abundant moisture the spores would form an irregular gelatinous group on the moist leaf surface. As the moisture disappeared the gelatinous walls would become thinner and the spores would be drawn closer together, finally forming a thin dry crust adhering tightly to the leaf surface.

Fresh material was sectioned by the free hand method, using 95 per cent alcohol to harden the material, sectioning the pith in alcohol, placing the sections in water and mounting the sections with the aid of a dissecting microscope in lactophenol and cotton blue. Photomicrographs of these sections are shown in figures 4-8. The striking feature of the free hand sections is the enormous length of the pedicels as they occur in the sorus. The spore in the upper right hand corner of figure 5 has a pedicel that measures $130 \times 28 \mu$. It is possible that the lactophenol may have caused some additional swelling. The general appearance of such spores has a striking similarity to the elongation of the ascus immediately preceding spore discharge.

In a young sorus (FIG. 7) various stages in the development of the pedicel may be seen; the pedicel is slender at first, enlarging slightly just below the mature spore, then gradually expanding and elongating until all the spores are raised considerably above the level of the sorus (FIG. 5). Two mature sori are shown in cross section in the photomicrograph in figure 4. The elongated, swollen, more or less curved pedicels are very conspicuous here. A portion of the upper sorus of figure 4 is shown in higher magnification in figure 6. The mature teliospore may now be discharged if the right conditions of moisture are obtained. The inner wall absorbs water slowly through the wall and through connecting hyphal cells. The resulting internal pressure presumably ruptures the outer wall and the teliospore is discharged. The break usually occurs somewhere in the lower end of the pedicel: it may be just above the basal portion or higher; it never includes the basal portion. Thus in an old sorus that has discharged most of the spores, there remains a mass of torn pedicel bases which

gives the sorus a very ragged appearance, clearly shown in the photomicrograph in figure 8.

Since the pedicel probably requires free water for successful discharge an attempt was made to determine the effect of free water upon the mature sorus. A leaf with a mature sorus was placed under the high power of a binocular dissecting microscope. At this magnification and with good illumination the black, shiny teliospores could be clearly observed. The sorus was then flooded with water. Almost immediately there were signs of activity as evidenced by slight movements of spores. These movements were mostly from side to side but soon it was evident that there was a strong upward movement as well. In a few minutes all of the spores were exhibiting a twisting motion that was fascinating to observe. The spores were thus pushed upward into the drop of water. No sudden discharge was observed under these conditions although many spores became free in the drop of water.

Leaves bearing telia were placed in Petri dishes and slides suspended a short distance (2-4 mm.) above the sori. In some cases teliospores were found on the lower surface of the slide suggesting that they had been deposited there by spore discharge. The leaves, however, are not flat and are inclined to curl so that it is possible that in some cases the leaf actually touched the slide. In one experiment four pedicellate spores were found at the end of twelve hours on one slide above a heavily infected leaf. At the end of twenty-four hours over 300 were present. One series of seven Petri dishes showed teliospores on three slides at the end of twenty-four hours. Another series of seven was left for four days. At the end of that time all of the slides had spores on their lower surface: three had many, two had four to six, whereas the remainder had but one teliospore each. In six of the seven dishes urediospores were present also on the slides, which means either that the slide accidentally touched the leaf or that the urediospores had also been discharged. Since the uredia are not known to discharge their spores it suggests accidental contact.

The lower surface of the exposed slides was not coated with an adhesive, yet the teliospores were firmly attached. The teliospore itself is apparently adhesive, sticking to any surface. In the

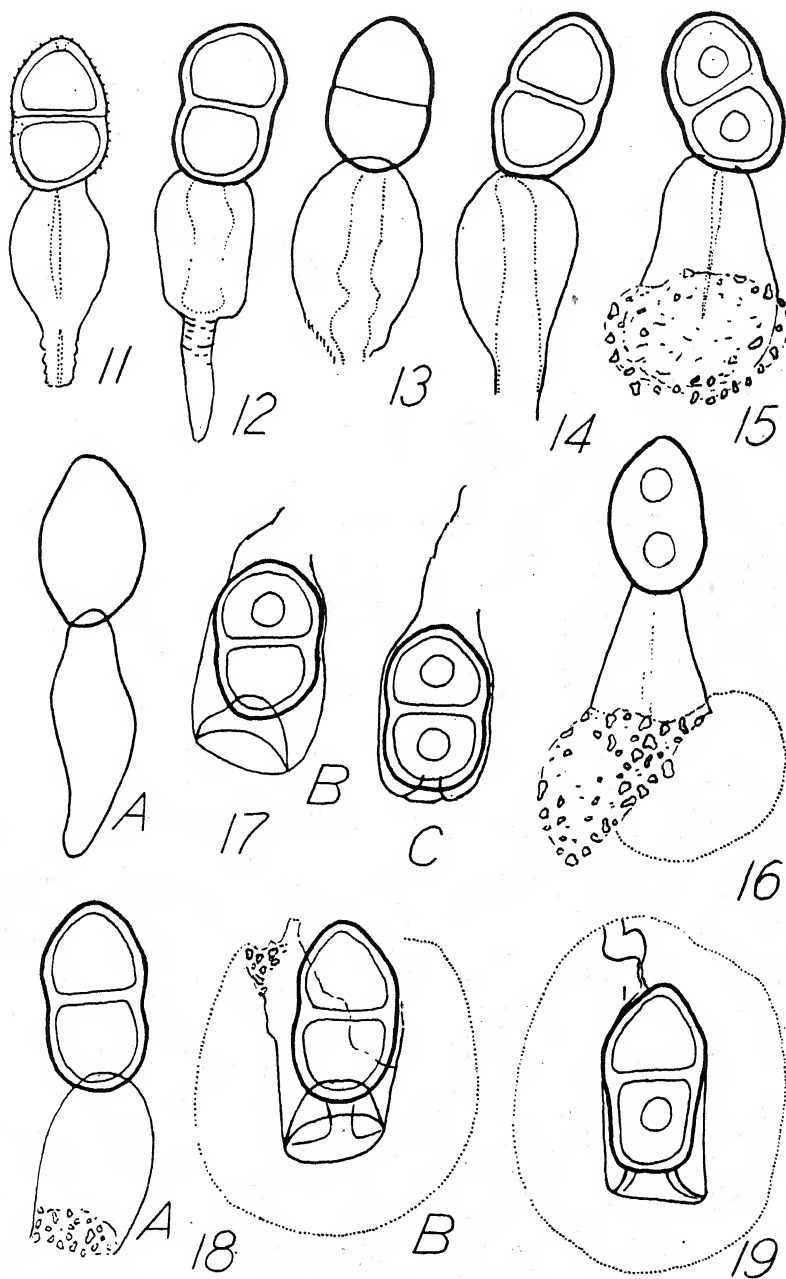
Basidiomycetes all discharged spores have been found to have sticky or moist surfaces. This would explain the adhesiveness of the discharged teliospores, but would not account for the presence of the urediospores there unless they too had a sticky surface.

DISCUSSION

The teliospore pedicel in the rusts not uncommonly swells when moistened in water. In *Phragmidium* this is the basis upon which the various sections are separated: in the *Euphragmidium* section "the lower half of the pedicel is hygroscopic" whereas in the section Earlea the pedicel is "firm not hygroscopic."³ Pedicel swelling also occurs in *Gymnosporangium inconspicuum* and the pedicel in *G. claviceps* is very similar to the pedicel of *P. tumidipes*. In *Uropyxis amorphae* a very unusual situation is found in which the teliospore is hygroscopic as well as the pedicel. The teliospore has an "inner layer chocolate-brown 2.5–3 μ thick, the outer layer colorless, gelatinous, swelling in water 7–15 μ thick at sides and 1–3 μ thick at apex and base, finally bursting and disappearing; pedicel colorless, as long as the spore, deciduous or swelling in water and bursting."³ If the pedicel bursts in water it is also probable that bursting occurs in the sorus and that discharge of the teliospores could take place. Three species of *Puccinia* are recorded³ in which the pedicel swells in water: *P. dondiae* on *Suaeda intermedia*, *P. globosipes*⁵ on *Lycium andersonii* and other species, and *P. tumidipes*. *Puccinia globosipes* is probably very closely related to *P. tumidipes*.

The discharge of teliospores is not primarily a function of the pedicel, but under certain conditions the swelling causes the pedicel to rupture, but whether this is done with considerable force is not known. In many sori loose teliospores are very numerous on the surface of the sori suggesting that pedicel rupture has merely set them free. If sufficient moisture accumulated around the sorus a film would form on the lower surface and a drop would tend to develop at that point. Teliospores would be set free in this drop, and due to the position of the leaf would spread out over the sur-

⁵ *Puccinia grayiae* Arth. is listed in Arthur's Manual as having swollen pedicels. However this species is now considered to be synonymous with *P. globosipes* (G. B. Cummins: personal communication).



FIGS. 11-19. *Puccinia tumidipes*.

face to one side of the sorus. As the moisture evaporated the gelatinous mass of spores and pedicels would dry down to the flaky crust found on most dry leaves. The observations described earlier of the activity of teliospores when a drop of water was added suggest that this method of spore discharge into a drop of water may be very common.

Discharged teliospores are sometimes found in typical groups on leaves which bear no telial sori. Obviously these must have come from some other leaf but whether they came by way of a drop falling from one leaf to another or whether they were shot there through the air could not be determined.

The position of the pedicel in the discharged teliospore is of interest: in one count there were 134 spores in a group of which 120 were reversed and fourteen pedicellate. On the other hand some counts show as high as 75 per cent of the latter type. In the experiments where leaves were placed in Petri dishes and the spores were discharged on a slide the majority of the discharged teliospores had the normal unreversed type of pedicel. When these discharged teliospores are mounted dry on a slide and water added, the majority of the pedicels show an immediate reversal. This may be due simply to the extremely rapid imbibition of water by the inner layer of the pedicel wall in the region exposed to the water, namely the ruptured base. Rapid swelling ensues and tremendous pressure built up at that point results in the pedicel turning inside out and surrounding the spore. The conditions requisite for this would appear to be a discharged spore with ruptured pedicel, desiccation, followed by abundant moisture. Undischarged teliospores would be unable to do this as the intact pedicel with its narrow base effectively prevents rapid absorption of water. It would be of interest to examine other rusts whose pedicels swell in water, such as *Uropyxis amorphae*, to see whether or not there is a similar mechanism.

SUMMARY

Discharged teliospores of *Puccinia tumidipes* were found in the vicinity of the telia on the lower surface of *Lycium halimifolium*. The pedicels were discovered to have a highly hygroscopic inner layer which swells greatly in water rupturing the outer wall and

setting the spore free. Under certain conditions the pedicel reverses itself, completely surrounding the teliospore. The discharged spores are sticky, adhering to each other and to the leaf surface. This is the first report of teliospore discharge in the Uredinales.

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EXPLANATION OF FIGURES

FIGS. 1-3. Photographs of lower surface of leaves of *Lycium halimifolium* showing discharged teliospores in black, fan-shaped areas adjacent to telia.

FIG. 1 $\times 4$ (approx.).

FIGS. 2, 3 $\times 15$ (approx.).

FIGS. 4-8. Photomicrographs of freehand cross sections of fresh material showing mature teliospores with greatly elongated pedicels. Figure 7 is a section through a young telium; figure 8 is through an old telium and shows ruptured pedicel bases.

FIG. 9. Photomicrograph of discharged teliospores with reversed pedicels from leaf surfaces mounted in lactophenol and stained with cotton blue. Note lack of pedicel swelling in the reversed spores, and also the presence of a single pedicellate teliospore.

FIG. 10. Photomicrograph of discharged teliospores with reversed pedicel from leaf mounted in water, later replaced by India ink. Note enormous swelling of pedicel wall.

FIG. 4 reduced to $\times 40$ (approx.).

FIGS. 5-8 reduced to $\times 200$ (approx.).

FIGS. 9, 10 reduced to $\times 250$ (approx.).

FIG. 11. Normal teliospore and pedicel (from Arthur's Manual).

FIG. 12. Teliospore in lactophenol with intact pedicel. Hygroscopic layer shorter than normal.

FIGS. 13, 14. Discharged teliospores mounted in lactophenol. Note ruptured base. Inner margin of hygroscopic layer shown by dotted lines.

FIG. 15. Discharged pedicellate spore mounted in water showing swollen ruptured base with granulations.

FIG. 16. As in figure 15, hygroscopic layer bulging outward.

FIG. 17. *A*, discharged spore mounted dry; *B*, same a few seconds after water added, pedicel now reversed; *C*, same a minute later. Outer margin of pedicel not visible.

FIG. 18. *A*, discharged teliospore mounted dry. *B*, same spore a few seconds after water added. India ink used to determine outer margin of pedicel wall (shown by dotted lines).

FIG. 19. Teliospore from leaf surface completely surrounded by reversed pedicel. Gelatinous layer 18μ thick: total width 65μ .

FIGS. 12-19 drawn by camera lucida. Magnification $\times 520$.

ASSAY OF CELLULOLYTIC ACTIVITY OF MOLDS ISOLATED FROM FABRICS AND RELATED ITEMS EXPOSED IN THE TROPICS *

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KATHRYN SANDERSON

(WITH 3 FIGURES)

INTRODUCTION

Cotton fabrics exposed in nature are subject to deteriorating action by a number of factors, chiefly sunlight, fungi, and bacteria. These and others of lesser overall importance interact in a complicated pattern at a rate depending upon the nature of the fabric itself and upon the particular set of environmental circumstances attending the exposure. The nature of the past war was such that fabrics in immense quantity were exposed for long periods to the degrading influence of intense tropical sunlight, to high humidity and wetness which favored fungus action, or to actual drenching by rain and contact with soil thus introducing possible bacterial action into the picture; or in any given case a fabric may have been exposed alternately or concurrently to two or more such factors. The destructive action of fungi was especially marked in the Southwest Pacific and in the China-Burma-India region.

This soon led to an intense program of "tropic-proofing," first perhaps in Australia, to be followed closely by responsible organizations in the United States, Canada, England, India, and New Zealand. The work, at first largely empirical, became gradually more fundamental as the need for more knowledge concerning the basic nature and cause of the deterioration became increasingly apparent. Late in 1944, the Quartermaster Corps undertook a pro-

* Extract presented at the Meeting of the American Association for the Advancement of Science, Boston, Dec. 1946; abstract in *Amer. Journ. Bot.* 33: 832-833. 1946.

gram of basic research into the causes and processes involved in the deterioration of fabrics and like materials. The object of this program was to provide a basis for the production of field items better qualified to withstand such adverse conditions.

One of the first phases of the broad program was a study of the nature of the fungous flora of fabrics deteriorated in the tropics. Many hundreds of cultures were accumulated from a wide range of tropical regions. These came through a variety of channels, but the large majority of those included in the present paper were isolated at this laboratory during the winter of 1944-45 by Dr. Elwyn T. Reese. These are designated by "PQMD" preceding the number. Nearly all of them were made from samples from the Southwest Pacific or India. These had been sent from the field accompanied by certain essential data and wrapped to specification to preclude contamination in transit. Several isolation procedures were used in order to select not only those organisms capable of cellulolytic action but also a fair sampling of others present. In the aggregate the cultures that have been assembled have provided what may be considered a good background of knowledge of the mycobiota of tropical fabrics as a potentiality. An evaluation of the behavior and action and relative importance of each species in nature is another matter requiring laboratory work with complementing skillful field observation over a considerable period of time.

OBJECTIVES AND LIMITATIONS OF THE PRESENT PAPER

The purpose of this paper is to report the results of a series of routine assays for cellulolytic activity of some of the fungi isolated from military cotton fabrics and related materials which had undergone deterioration in the tropics. They were conducted for the purpose of separating the cellulolytic from the non-cellulolytic species and to provide a fair conception of the comparative rapidity of action. The results are to be considered preliminary and suggestive, an early step toward an understanding of the nature and activity of the mycobiota of fabrics and similar materials exposed under unfavorable conditions. Certain cultures have been selected for more intensive study and for use in the biochemical phases of the Quartermaster program.

A really meaningful comparison of the cellulolytic activity of strains or species or organisms can be made only after the particular set of cultural conditions optimal for each entity is known. A relatively full understanding of the requirements and behavior of an organism with regard to its cellulolytic activity in pure culture is essential if the organism is to be used in the experimental work of a program of fundamental research directed toward the development of biologically resistant materials. The establishment of a species as a "strong" or "weak" cellulose destroyer under laboratory conditions must not, however, be interpreted empirically as an index to the economic importance of the particular species in the deterioration of fabrics under conditions of field exposure. For example there is as yet no evidence that *Myrothecium verrucaria* (= *Metarrhizium glutinosum*), which perhaps is the most strongly cellulolytic species of fungus yet discovered, and therefore widely used in laboratory experimental work, is of any significance at all in the destruction of fabrics in the field.

Conversely the demonstration that the members of a species are incapable of direct enzymatic action against the basic cellulosic ingredients of a fabric does not mean that such a species may be dismissed as of no significance. Much depends upon the nature of the fabric and its intended use. In the destruction of waterproofing or various coating materials a fabric may be rendered unserviceable without direct cellulolytic action, or the way thus may be opened for the later cellulolytic attack. In this connection it may be noted that in the prewar years when the British Cotton Industry found itself faced with numerous claims for mildew damage to cotton goods in transit to Eastern markets it was found that the main problem was that of controlling fungi which grow in the starchy material used in sizing. Galloway (2), in a statement of the problems confronting the industry, listed the objectionable effects of fungi as (1) musty smell, (2) colored stains, (3) production of acids and alkali, and (4) tendering or actual enzymatic destruction of the fibers. Staining, which included both pigment production and the coloration of the fungus itself, was said to be by far the most important effect and the cause of the majority of damage claims.

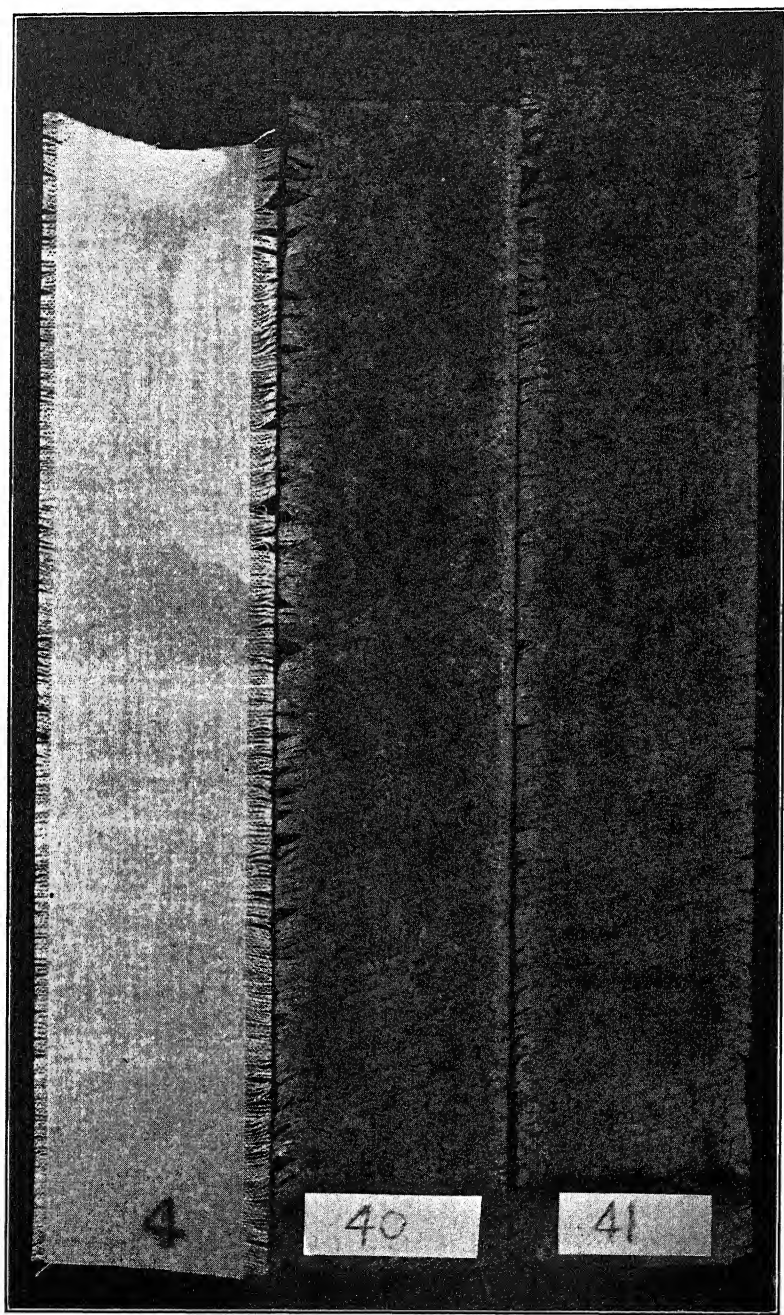


FIG. 1. Assay of cellulolytic activity.

Many organisms commonly isolated from fabrics are there only in the form of spores deposited with the dust of the atmosphere and are incapable of any direct action against the basic ingredients of the fabric, whereas others potentially destructive are probably seldom operative in nature. There is reason to believe that continued work will eventually demonstrate that a large portion of the actual decay of the fibers, especially under a defined range of environmental factors, is due to a relatively small number of species of fungi.

A review of pertinent literature will not be undertaken, for it would be of little value unless it could be more critical than space will permit in this paper. That appearing prior to 1927 was summarized by Thaysen and Bunker (13), and enough of the publications appearing since that time to provide an entrance to the literature are listed at the end of the present paper.

A more detailed analysis of the identity, distribution, specificity for materials, and economic significance of some of the species and groups of species should be forthcoming in future articles.

MATERIALS AND METHODS

The assays were conducted concurrently with other work extending over a period from October 1944 to December 1945. The basic method was that described recently by Greathouse, Klemme, and Barker (4) in which a spore suspension is placed on a strip of cotton fabric in the presence of a solution of mineral salts and incubated. The decline in tensile strength of the cloth was taken as a measure of the cellulolytic activity of the organism. The nutrient solution used was that designated as "Formula A" and had the following composition in grams per liter: K_2HPO_4 , 1.3940; $MgSO_4 \cdot 7H_2O$, 0.7395; NH_4NO_3 , 1.0006; $CaCO_3$, 0.005; $NaCl$, 0.005; $Fe_2(SO_4)_3 \cdot 7H_2O$, 0.001; $MnSO_4$, 0.001; and $ZnSO_4 \cdot 7H_2O$, 0.001. The initial pH was approximately 6.8.

In the course of the tests, several successive slight variations were introduced into the basic method. These are summarized briefly in Table I. When 16-oz. French square bottles were used (method variants 1-3), a $1\frac{1}{2}$ inch diameter glass fabric insert was used in the cap to provide for aeration. Forty milliliters of a

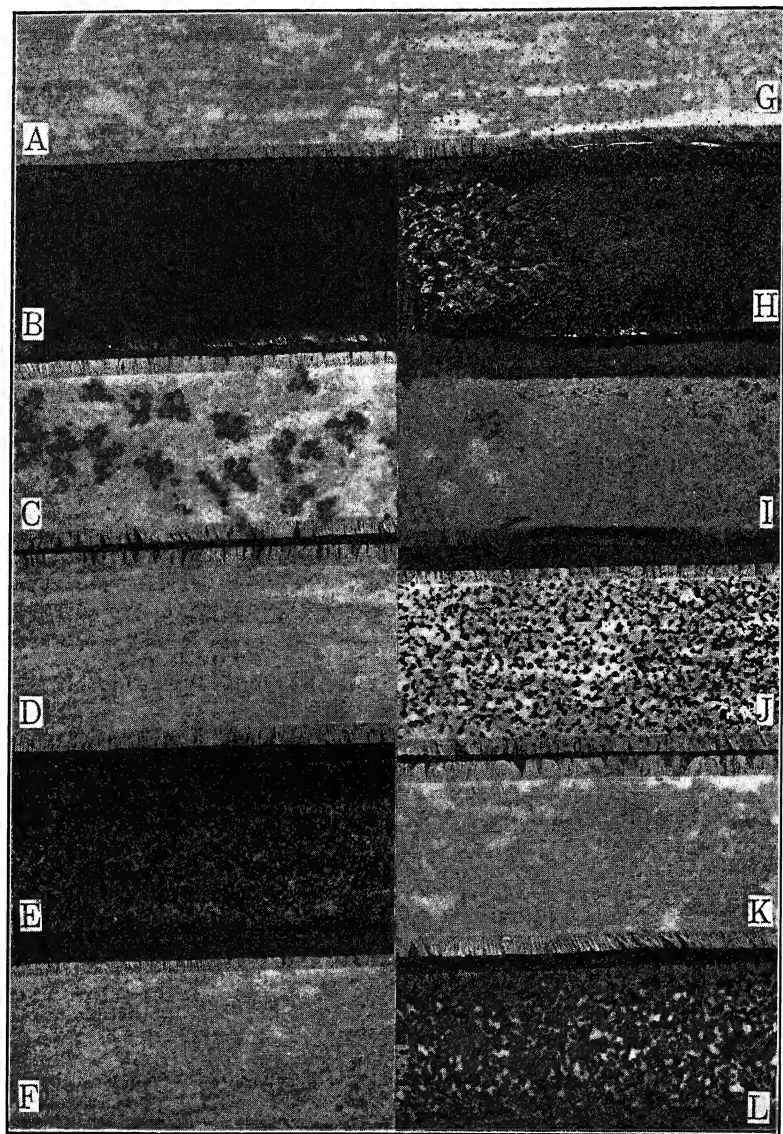


FIG. 2. Assay of cellulolytic activity.

medium consisting of the mineral salts plus 1.5 per cent agar was placed in each bottle, autoclaved for 20 minutes at 15 lbs. pressure, and the bottles laid on their sides to cool. The strips of fabric, cut to a length of approximately six inches and raveled to a width of exactly 1 inch, were subjected to similar autoclaving, dry, and

TABLE I
OUTLINE OF VARIATION IN BASIC METHOD USED FOR ASSAY FOR
CELLULOLYTIC-ACTIVITY OF FUNGI

Variant	Cloth		Incubation	
	Type	Tensile Strength*	Vessel	Temp.-humidity
1	4 oz. sheeting	55.2 ± 0.5 , $\sigma = 3.5$, $N = 87^*$	Bottle	Room
2	3.3 oz. sheeting	38.2 ± 0.2 , $\sigma = 1.6$, $N = 351$	Bottle	Room
3	3.3 oz. sheeting	38.2 ± 0.2 , $\sigma = 1.6$, $N = 351$	Bottle	85° F., 80% r.h.
4	3.3 oz. sheeting	38.2 ± 0.2 , $\sigma = 1.6$, $N = 351$	Test tube	85° F., 80% r.h.
5	Gray duck (a)	179.2 ± 2.1 , $\sigma = 5.6$, $N = 9$	Bottle	85° F., 80% r.h.
6	Gray duck (a)	179.2 ± 2.1 , $\sigma = 5.6$, $N = 9$	Test tube	85° F., 80% r.h.
7	Gray duck (b)	143.2 ± 1.5 , $\sigma = 12.2$, $N = 65$	Test tube	85° F., 80% r.h.
8	12.29 oz. gray duck	169.3 ± 3.8 , $\sigma = 15.0$, $N = 17$	Test tube	85° F., 80% r.h.

* Tensile strength is expressed as the arithmetic mean of a number of replicates (N) in pounds.

σ = Standard deviation $\frac{\sqrt{\sum X^2}}{N}$ where X is the deviation of the individual values from the mean.

in a separate container. They were then transferred under sterile conditions by means of a long pair of forceps one to each bottle and placed flat on the agar. Strips of 3.3 oz. bleached cotton sheeting incubated in accordance with this procedure are shown in figures 1 and 2. When test tubes were employed (method variants 4–8), the size used was 150×25 mm., of 75 ml. capacity. Twenty-five milliliters of the salts solution was placed in each and the cloth strip inserted. Slightly less than the lower half of the strip was submerged. The tubes were then plugged with cotton, sterilized as above, and allowed to cool. The method is illustrated in figure 3.

The variations in procedure outlined in Table I should be taken into consideration in interpreting the results. There is evidence that some organisms capable of attacking gray duck were, due

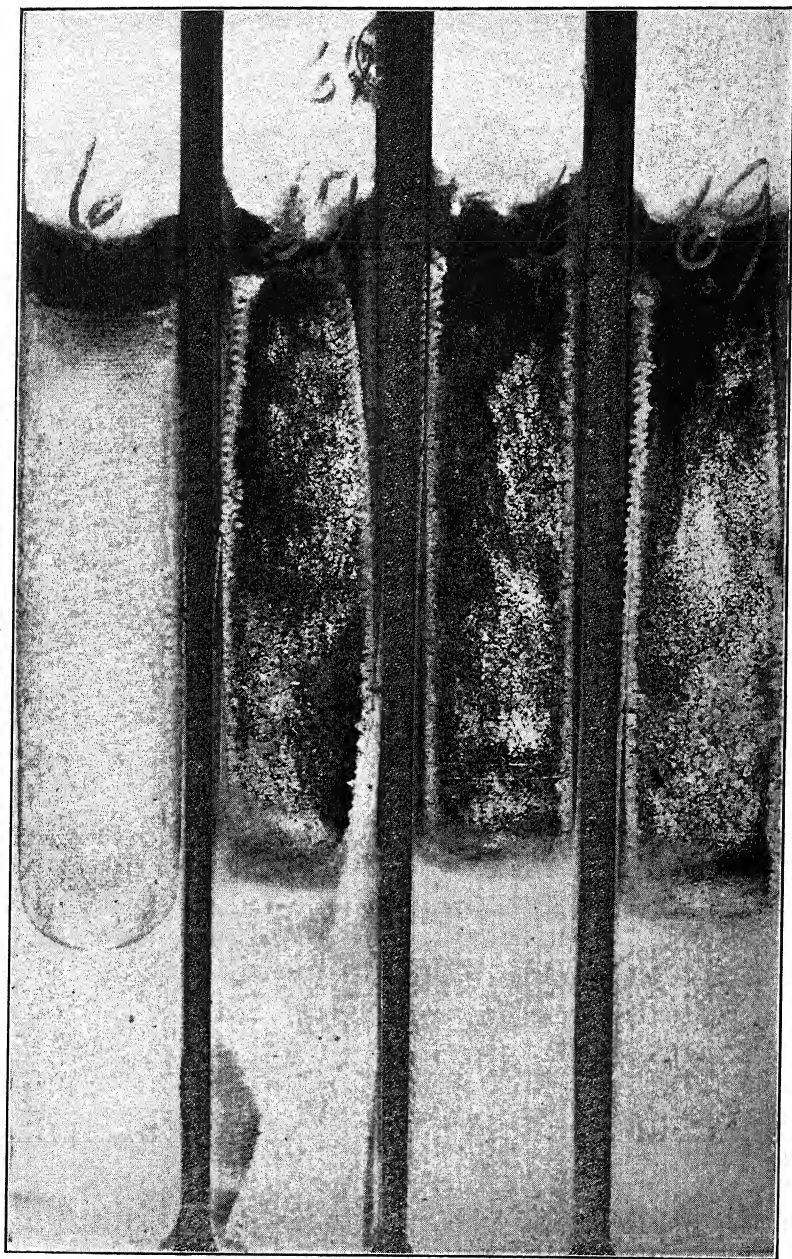


FIG. 3. *Aspergillus luchuensis* on gray cotton duck.

probably to nutritive deficiencies, incapable of cellulolytic action against the more highly purified bleached sheeting. Aside from this, there are few, if any instances where the variation in method meant a difference of positive or negative results.

The inoculum in all cases was prepared by growing the fungi on potato dextrose agar slants (20 g. dextrose per liter) under room conditions until good sporulation resulted. For most species a period of two to three weeks was found to be about right, although a few required a considerably longer period. The tube was then filled nearly to the top with sterile distilled water, the surface of the slant scraped with the tip of a 2 ml. pipette until a heavy spore suspension was obtained, and 2 ml. of the resulting suspension was distributed evenly over the surface of the cloth by means of the pipette.

When the bottle method was used the fungi were killed at the end of the incubation period by inverting the bottles, placing in each a few drops of formalin, replacing the ventilation caps with solid ones, and allowing to stand over night. When test tubes were used the strips were removed and washed in 70 per cent alcohol. In either case the strips were rinsed finally in tap water, dried for a few hours under room conditions, subjected to standard conditioning (24 hours at 70° F. and 65 per cent relative humidity), and broken on a motor driven Scott tester with a 3-inch space between the jaws.

About 20 cultures were assayed at a time. With each such lot were included two sets of controls, one on which was placed 2 ml. of distilled water, and the other inoculated with *Chaetomium globosum*, USDA 1042.4, as a standard for comparison.

Culture numbers are preceded by a prefix indicating the institution at which the isolation was made, as follows:

PQMD. A series of isolations started by Prof. W. H. Weston at Harvard University in June 1944 and continued at the Philadelphia Quartermaster Depot by Dr. Elwyn T. Reese through the first half of 1945. Nearly all that are included in this paper are from items collected in the Southwest Pacific or India.

JQMD. Isolations made at the Jeffersonville (Indiana) Quartermaster Depot by, or under the direction of, Dr. G. W. Martin

or Dr. W. D. Gray from similar materials and over a similar period.

Fla. Isolations by Dr. G. F. Weber at University of Florida from Quartermaster items exposed experimentally at Camp Indian Bay, Florida.

Aust. Isolates made during the early period of the war in the Pacific by the Australian Mycological Panel, mostly from military items collected in New Guinea.

MIT. Isolates made during the early months of 1944 at the Chemical Warfare Service Development Laboratory, Massachusetts Institute of Technology, from items under test in local "tropical" chambers and from the tropics.

42nd Chem. Co. Isolates made in the Southwest Pacific by the 42nd Chemical Laboratory Co. of the Chemical Warfare Service in 1943 and sent to Chemical Warfare Service Development Laboratory, M.I.T.

USDA. Cultures obtained from the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture—mostly of local origin.

RESULTS

The results are presented in Table II.

The figures cited for the inoculated strips are based on three to five replicates. The uninoculated controls from the several experiments were combined to arrive at the arithmetic means used as 100 per cent. Analysis of these figures indicates that the dispersion of the individual values around the arithmetic mean is quite large. Thus, for the 3.3 oz. cloth the 3σ limits are about 19 per cent and for the 4.0 oz. cloth about 12.5 per cent. In view of such wide dispersion and the fact that a maximum of five replicates were used for any given percentage figure, it is probably reasonable to assume that any decline in tensile strength less than 20 per cent is of doubtful significance.

TABLE II
ASSAY OF FUNGI FOR CELLULOLYTIC ACTIVITY

Species	Culture Number	Source ¹	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Aspidia capillata</i> van Tieg.	PQMD 8b	Undershirt	Bougainville Is.	3	99	102	105		0
{ <i>Acladium</i> sp.?}	PQMD 45f	Shoe	Ledo, India	2	42	31	18		4
	PQMD 45f	Shoe	Ledo, India	3	38	16	7		4
	PQMD 49c	Web belt	Ledo, India	2	52	39	31		4
<i>Acremonium</i> sp.	PQMD 1b	Tent	Bougainville Is.	1		37	24		3
<i>Acremonium</i> sp.	PQMD 1b	Tent	Bougainville Is.	7		41	25		4
	PQMD 121j	Tarpaulin	Panama	3	43	32	29	17	2
<i>Alternaria tenuis</i> auct.	PQMD 26a	Canvas	Russell Is.	2	94	79	65		3
<i>Alternaria tenuis</i> auct.	PQMD 73b	Plastic canteen	Finsch., N.G.	3	80	76	70		2
<i>Alternaria tenuis</i> auct.	Fla B-7	Tent	Florida	3	82	79	59		3
<i>Alternaria tenuis</i> auct.	Fla B-47	Canvas	Florida	3	76	66	59		2
<i>Alternaria tenuis</i> auct.	Fla B-48	Canvas	Florida	3	74	67	55		4
<i>Arthrosporium</i> sp.	PQMD 124f	Duck	Panama	3	108	106	101		0
<i>Aspergillus Chevalieri</i> (Mang.) Th. & Ch.	PQMD 52d	Wool-cotton shirt	Finsch., N.G.	2	104	106	113		0
<i>Aspergillus Chevalieri</i> (Mang.) Th. & Ch.	PQMD 53d	Leather strap	Finsch., N.G.	2	105	100	102		0
<i>Aspergillus Chevalieri</i> (Mang.) Th. & Ch.	PQMD 59c	Canvas kit	Finsch., N.G.	2	107	103	102		0

¹ Designation of item from which organism was isolated is shortened as much as possible; in all cases except where obvious or otherwise designated, the base material is cellulose.

² Brackets so used indicate that the numbers included represent but one species.

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Aspergillus Chevalieri</i> (Mang.) Th. & Ch. (cont.)	PQMD 64c	Shirt	Finsch., N.G.	3	100	101	96		0
<i>A. Clavuligeri</i> var. <i>intermedius</i> Th. & Raper	PQMD 58b	Leatherette	Finsch., N.G.	2	104	101	101		0
<i>A. Fischeri</i> Wehm.	Fla A-11		Florida	1		87	62	40	3
<i>A. Fischeri</i> Wehm.	Fla A-11		Florida	4	68			0	2
<i>A. Fischeri</i> Wehm.	Fla A-11		Florida	7	91			36	4
<i>A. flavipes</i> (Bain & Sart.) Th. & Ch.	PQMD 24a	Netting	Russell Is.	1	63	56	47		4
<i>A. flavipes</i> (Bain & Sart.) Th. & Ch.	Fla A-14		Florida	2	86	68	55		4
<i>A. flavipes</i> (Bain & Sart.) Th. & Ch.	Fla B-67	Leggings	Florida	3	91	75	69		4
<i>A. flavus</i> Link	PQMD 4m	Shoe	Bougainville Is.	1	103	95	93	98	1
<i>A. flavus</i> Link	PQMD 10e	Canvas	Oro Bay, N.G.	1		91	98	91	0
<i>A. flavus</i> Link	PQMD 13a	Canvas	Espiritu Santo Is.	1		92	101	95	1
<i>A. flavus</i> Link	PQMD 13c	Canvas	Espiritu Santo Is.	8		101	98		3
<i>A. flavus</i> Link	PQMD 45c	Shoe	Ledo, India	2	102	97	107		0
<i>A. flavus</i> Link	PQMD 48d	Tent	Ledo, India	2	102	105	99		1
<i>A. flavus</i> Link	PQMD 63c	Rope	Finsch., N.G.	2	110	105	97		0
<i>A. flavus</i> Link	PQMD 70a	Shoe	Finsch., N.G.	3	94	101	91		0
<i>A. flavus</i> Link	PQMD 73a	Plastic canteen	New Guinea	3	99	95	99		0
<i>A. flavus</i> Link	PQMD 91a	Canvas	Finsch., N.G.	6	113			112	1
<i>A. flavus</i> Link	PQMD 91a	Canvas	Finsch., N.G.	7	102			107	0

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>A. flavus</i> Link (cont.)	JQMD 189	Tent	Hollandia, N.G.	3	105	97	95		1
<i>A. flavus</i> Link	Fla A-5		Florida	1		109	103	101	0
<i>A. flavus</i> Link	Fla A-6		Florida	1		105	98	111	1
<i>A. flavus</i> Link	Fla A-7	Canteen cover		1		109	101	111	1
<i>A. flavus</i> Link	Fla B-20	Shoe	Florida	3	93	99	93		0
<i>A. flavus</i> Link	Aust 3		New Guinea	3	100	96	91		0
<i>A. fumigatus</i> Fres.	PQMD 6b	Tent	Bougainville Is.	1		49	27	19	4
<i>A. fumigatus</i> Fres.	PQMD 6b	Tent	Bougainville Is.	4	81			16	3
<i>A. fumigatus</i> Fres.	PQMD 6b	Tent	Bougainville Is.	7	110			36	4
<i>A. fumigatus</i> Fres.	PQMD 45h	Shoe	Ledo, India	2	92	92	76		3
<i>A. fumigatus</i> Fres.	PQMD 45h	Shoe	Ledo, India	7		93		41	4
<i>A. fumigatus</i> Fres.	PQMD 45h	Shoe	Ledo, India	4		87		72	2
<i>A. fumigatus</i> Fres.	PQMD 91d	Canvas	Finsch., N.G.	3	84	63	44		4
<i>A. fumigatus</i> Fres.	PQMD 206	Tarpaulin	Cairo, Egypt	3	99	92	99		2
<i>A. fumigatus</i> Fres.	MIT 3c	Gas mask cover	India	3	57	26	16		4
<i>A. fumigatus</i> series	Fla B-21	Canteen cover	Florida	3	93	96	97		1
<i>A. fumigatus</i> series	Fla B-26	Cloth	Florida	3		97	89		1
<i>A. japonicus</i> Saito	PQMD 124g	Duck	Panama	3	100	95	96		0
<i>A. luchuensis</i> Inui	PQMD 23b	Rope	Port Moresby, N.G.	4		106		98	0
<i>A. luchuensis</i> Inui	PQMD 23b	Rope	Port Moresby, N.G.	7		108		89	3
<i>A. luchuensis</i> Inui	JQMD 745	Tent	Port Moresby, N.G.	4	92			97	0

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>A. luchuensis</i> Inui (cont.)	JQMD 745	Tent	Port Moresby, N.G.	4				98	0
<i>A. luchuensis</i> Inui	JQMD 745	Tent	Port Moresby, N.G.	7				78	4
<i>A. luchuensis</i> Inui	JQMD 745	Tent	Port Moresby, N.G.	7			73		4
<i>A. luchuensis</i> Inui	JQMD 745	Tent	Port Moresby, N.G.	6			68		4
<i>A. luchuensis</i> Inui	JQMD 745	Tent	Port Moresby, N.G.	7	103			92	3
<i>A. luchuensis</i> Inui	JQMD 745	Tent	Port Moresby, N.G.	6	75			49 ¹	4
<i>A. luchuensis</i> Inui	JQMD 745	Tent	Port Moresby, N.G.	5	94	82	77		3
<i>A. nidulans</i> (Eid.) Wint.	Fla A-13		Florida	4		93		72	2
<i>A. nidulans</i> (Eid.) Wint.	Fla A-13		Florida	7		105		106	2
<i>A. niger</i> group	PQMD 4j	Shoe	Bougainville Is.	1	106	93	90		1
<i>A. niger</i> group	PQMD 4j	Shoe	Bougainville Is.	4	92			102	1
<i>A. niger</i> group	PQMD 4j	Shoe	Bougainville Is.	5	107	98	102		2
<i>A. niger</i> group	PQMD 4j	Shoe	Bougainville Is.	7	107			115	1
<i>A. niger</i> group	PQMD 10c	Canvas	Oro Bay, N.G.	1	94	103	94		1
<i>A. niger</i> group	PQMD 10c	Canvas	Oro Bay, N.G.	4	94			105	0
<i>A. niger</i> group	PQMD 10c	Canvas	Oro Bay, N.G.	7	114			110	1
<i>A. niger</i> group	PQMD 10c	Canvas	Oro Bay, N.G.	5	103	102	105		2
<i>A. niger</i> group	PQMD 17g	Tent	Espiritu Santo Is.	1			94	100	1

¹ Figure 3.

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>A. niger</i> group (cont.)	PQMD 17g	Tent	Espiritu Santo Is.	4	102			102	0
<i>A. niger</i> group	PQMD 17g	Tent	Espiritu Santo Is.	5	103				2
<i>A. niger</i> group	PQMD 17g	Tent	Espiritu Santo Is.	7	111	100	95	112	0
<i>A. niger</i> group	PQMD 22c	Tarpaulin	Russell Is.	4	86			102	0
<i>A. niger</i> group	PQMD 22c	Tarpaulin	Russell Is.	7	112			108	0
<i>A. niger</i> group	PQMD 23f	Tarpaulin	Espiritu Santo Is.	1	92	98	105		2
<i>A. niger</i> group	PQMD 23f	Tarpaulin	Espiritu Santo Is.	4		106		101	0
<i>A. niger</i> group	PQMD 23f	Tarpaulin	Espiritu Santo Is.	7		110		110	1
<i>A. niger</i> group	PQMD 23f	Tarpaulin	Espiritu Santo Is.	7		103		111	0
<i>A. niger</i> group	PQMD 24d	Netting	Russell Is.	4		109			1
<i>A. niger</i> group	PQMD 24d	Netting	Russell Is.	7		103	105		2
<i>A. niger</i> group	PQMD 25a	Shoe	Russell Is.	1	100				0
<i>A. niger</i> group	PQMD 25a	Shoe	Russell Is.	4		108		103	0
<i>A. niger</i> group	PQMD 25a	Shoe	Russell Is.	7		106		112	1
<i>A. niger</i> group	PQMD 26f	Canvas	Russell Is.	4		103		103	0
<i>A. niger</i> group	PQMD 26f	Canvas	Russell Is.	7		110		109	1
<i>A. niger</i> group	PQMD 30a	Shoe	Kauai, T.H.	2	99	99	99	108	1
<i>A. niger</i> group	PQMD 30a	Shoe	Kauai, T.H.	4		108		108	1
<i>A. niger</i> group	PQMD 30a	Shoe	Kauai, T.H.	7		106			1
<i>A. niger</i> group	PQMD 34c	Shoe	Kauai, T.H.	1	105	101 ¹	105	101	2
<i>A. niger</i> group	PQMD 34c	Canteen cover	New Guinea	4		103		112	1
<i>A. niger</i> group	PQMD 34c	Canteen cover	New Guinea	7		108			1
<i>A. niger</i> group	PQMD 36b	Tarpaulin	New Guinea	2	107	102	105	108	0
<i>A. niger</i> group	PQMD 36b	Tarpaulin	New Guinea	4		101		103	1
<i>A. niger</i> group	PQMD 36b	Tarpaulin	New Guinea	7		103			0
<i>A. niger</i> group	PQMD 38b	Tentage	Karachi, India	2	102	105	102		1
<i>A. niger</i> group	PQMD 45d	Shoe	Ledo, India	2	94	99	102		0
<i>A. niger</i> group	PQMD 45d	Shoe	Ledo, India	4		103		101	0
<i>A. niger</i> group	PQMD 45d	Shoe	Ledo, India	7		104		99	2

¹ Figure 2G.

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>A. niger</i> group (cont.)									
<i>A. niger</i> group	PQMD 50c	Rope	Hawaii	2	99	105	97	98	1
<i>A. niger</i> group	PQMD 50c	Rope	Hawaii	4		106		106	0
<i>A. niger</i> group	PQMD 50c	Rope	Hawaii	7		105			1
<i>A. niger</i> group	PQMD 51c	Tent	Hawaii	2	107	105	102		1
<i>A. niger</i> group	PQMD 64b	Shirt	Finsch., N.G.	2	101	101	97		0
<i>A. niger</i> group	PQMD 95b	Shoe	Florida	4	89			102	0
<i>A. niger</i> group	PQMD 95b	Shoe	Florida	7	111			114	1
<i>A. niger</i> group	PQMD 104b	Rubber boot	Finsch., N.G.	4	84			105	0
<i>A. niger</i> group	PQMD 104b	Rubber boot	Finsch., N.G.	7	111			108	0
<i>A. niger</i> group	PQMD 106f	Legging	Finsch., N.G.	4	97			102	0
<i>A. niger</i> group	PQMD 108d	Shoe	Florida	7	112			109	1
<i>A. niger</i> group	PQMD 108d	Shoe	Florida	4	86			102	0
<i>A. niger</i> group	PQMD 108d	Shoe	Florida	7	107			106	1
<i>A. niger</i> group	Fla A-1a	Tent	Florida	1		103	94	103	1
<i>A. niger</i> group	Fla A-2		Florida	1		103	100	105	1
<i>A. niger</i> group	Fla B-27	Cloth	Florida	3	97	99	104		1
<i>A. niger</i> group	Fla B-55	Tent	Florida	3	96	101	99		1
<i>A. niger</i> group	Fla C-98	Typewriter ribbon	Florida	3	102	104	99		1
<i>A. niger</i> group	Aust 26	Electrical	Australia	7		98		101	1
<i>A. niger</i> group	Aust 26	Electrical	Australia	4		108		106	1
<i>A. niger</i> group	42nd Chem. 19b			4		101		108	0
<i>A. niger</i> group	42nd Chem. 19b			7		101		99	1
<i>A. ochraceus</i> Wilh.	PQMD 26b	Canvas	Russell Is.	2	99	105	97	103	1
<i>A. ochraceus</i> Wilh.	PQMD 26b	Canvas	Russell Is.	4		103		98	0
<i>A. ochraceus</i> Wilh.	PQMD 26b	Canvas	Russell Is.	7		105			1
<i>A. ochraceus</i> Wilh.	PQMD 58c	Leatherette	Finsch., N.G.	2	100	92	92		2

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>A. Oryzae</i> (Ahl.) Cohn	PQMD 22b	Canvas	Russell Is.	4	86		102		0
<i>A. Oryzae</i> (Ahl.) Cohn	PQMD 22b	Canvas	Russell Is.	7	117		110		1
<i>A. Oryzae</i> (Ahl.) Cohn	42nd Chem. 18a	Gas mask cover	Milne Bay, N.G.	3	106	93	94		0
<i>A. repens</i> (Corda) DeBary	PQMD 44c	Tobacco	S.W. Pacific	2	105	99	102		0
<i>A. repens</i> (Corda) DeBary	PQMD 56f	Rope	Finsch., N.G.	2	102	100	104		1
<i>A. repens</i> (Corda) DeBary	PQMD 56f	Rope	Finsch., N.G.	3	101	98	94		0
<i>A. repens</i> (Corda) DeBary	PQMD 56f	Rope	Finsch., N.G.	7		99	103	97	0
<i>A. repens</i> (Corda) DeBary	PQMD 58h	Leatherette	Finsch., N.G.	2	102	102	99		0
<i>A. repens</i> (Corda) DeBary	PQMD 59g	Canvas	Finsch., N.G.	2	84	98	91		0
<i>A. repens</i> (Corda) DeBary	PQMD 59g	Canvas	Finsch., N.G.	3	84	97	91		0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 4d	Shoe	Bougainville Is.	1		100	103		1
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 4d	Shoe	Bougainville Is.	1	110	102	103		1
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 4d	Shoe	Bougainville Is.	4		106		95	0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 4d	Shoe	Bougainville Is.	7		101		109	1
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 26h		Bougainville Is.	7		100	102	95	1
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 31c	Canvas	New Guinea	1	100	105	103		2
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 39a	Canvas	Karachi, India	2	99	102	102		1
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 41a	Rope	Karachi, India	2	107	107	105		0

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch. (cont.)	PQMD 48e	Tent	Ledo, India	2	97	86	84		3
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 54a	Tent	Finsch., N.G.	2	104	104	102		0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 55e	Leather	Finsch., N.G.	2	103		104		0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 58d	Leatherette	Finsch., N.G.	2	106	101	106		2
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 58e	Leatherette	Finsch., N.G.	2	99	107	102		0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 58g	Leatherette	Finsch., N.G.	2	109	99	99		0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 58g	Leatherette	Finsch., N.G.	2	97	103	104		0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 60a	Leatherette	Finsch., N.G.	2	109	100	105		1
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 61a		Finsch., N.G.	2	103	103	98		0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 62d	Corncob pipe	Finsch., N.G.	2	100	103	99		0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 62f	Corncob pipe	Finsch., N.G.	2	107	104	93		0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	PQMD 73c	Plastic canteen	Finsch., N.G.	3	96	93	94		0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	Fla B-18	Canteen cover	Florida	3	98	99	89		0
<i>A. Sydowi</i> (Bain. & Sart.) Th. & Ch.	Fla B-42	Canvas	Florida	3	88	103	100		0

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>A. Tamarit</i> Kita	PQMD 9a	Cellophane	Oro Bay, N.G.	1		89	101	87	2
<i>A. Tamarit</i> Kita	PQMD 9a	Cellophane	Oro Bay, N.G.	4	105			102	0
<i>A. Tamarit</i> Kita	PQMD 9a	Cellophane	Oro Bay, N.G.	7	108			110	2
<i>A. Tamarit</i> Kita	PQMD 50b	Rope	Hawaii	2	99	105	105		1
<i>A. Tamarit</i> Kita	PQMD 51h	Tent	Hawaii	2	107	105	94		1
<i>A. terreus</i> Thom	PQMD 72f	Leather	Finsch., N.G.	3	67	42	32		4
<i>A. terreus</i> Thom	PQMD 91c	Canvas	Finsch., N.G.	4	72			19	4
<i>A. terreus</i> Thom	PQMD 91c	Canvas	Finsch., N.G.	7	99			42	4
<i>A. terreus</i> Thom	PQMD 94b	Trousers	Finsch., N.G.	7			23		4
<i>A. terreus</i> Thom	PQMD 94b	Trousers	Finsch., N.G.	7			22		4
<i>A. terreus</i> Thom	PQMD 106g	Legging	Finsch., N.G.	4	47			16	3
<i>A. terreus</i> Thom	PQMD 106g	Legging	Finsch., N.G.	7	94	41	26	43	3
<i>A. terreus</i> Thom	PQMD 481	Cord	Jeffersonville, Ind.	3	63				4
<i>A. terreus</i> Thom	Fla B-19	Canteen cover	Florida	3	70	51	34		4
<i>A. terreus</i> Thom	MIT 7	Wood	Cambridge, Mass.	2	75	58	41		3
<i>A. unguis</i> (Em.-Weil & Gaud.) Th. & Rap.	PQMD 8f	Undershirt	Oro Bay, N.G.	1		103	96	96	1
<i>A. unguis</i> (Em.-Weil & Gaud.) Th. & Rap.	PQMD 8f	Undershirt	Oro Bay, N.G.	1		87	103	92	1
<i>A. unguis</i> (Em.-Weil & Gaud.) Th. & Rap.	PQMD 25b	Shoe	Russell Is.	1		103	96	100	1
<i>A. unguis</i> (Em.-Weil & Gaud.) Th. & Rap.	PQMD 30b	Shoe	Kauni, T.H.	2	97	105	107		0
<i>A. unguis</i> (Em.-Weil & Gaud.) Th. & Rap.	PQMD 45e	Shoe	Ledo, India	2	97	105	105		0

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>A. unguis</i> (Em.-Weil & Gaud.) Th. & Rap. (cont.)	PQMD 53c	Leather	Finsch., N.G.	2	110	100	103		0
<i>A. unguis</i> (Em.-Weil & Gaud.) Th. & Rap.	PQMD 64a	Shirt	Finsch., N.G.	2	102	98	100		0
<i>A. unguis</i> (Em.-Weil & Gaud.) Th. & Rap.	PQMD 65b	Canvas	Finsch., N.G.	2	99	102	94		0
<i>A. unguis</i> (Em.-Weil & Gaud.) Th. & Rap.	PQMD 102g	Canvas	Finsch., N.G.	4	90			98	0
<i>A. unguis</i> (Em.-Weil & Gaud.) Th. & Rap.	PQMD 102g	Canvas	Finsch., N.G.	7	110			109	0
<i>A. ustus</i> (Bain) Th. & Ch.	PQMD 4h	Shoe	Bougainville Is.	1	96	102	93		2
<i>A. ustus</i> (Bain) Th. & Ch.	PQMD 4h	Shoe	Bougainville Is.	4		72		41	1
<i>A. ustus</i> (Bain) Th. & Ch.	PQMD 4h	Shoe	Bougainville Is.	7		103		84	3
<i>A. ustus</i> (Bain) Th. & Ch.	PQMD 51g	Tent	Hawaii	2	107	105	107		1
<i>A. ustus</i> (Bain) Th. & Ch.	PQMD 57c	Imitation leather	Finsch., N.G.	2	104	93	89		3
<i>A. ustus</i> (Bain) Th. & Ch.	IQMD 272			3	93	93	93		2
<i>A. ustus</i> (Bain) Th. & Ch.	Fla A-12		Florida	1		100	83	63	4
<i>A. ustus</i> (Bain) Th. & Ch.	Aust 22	Silk	New Guinea	3	90	70	69		3
<i>A. ustus</i> var. <i>laevis</i> (Block.) Th. & Rap.	PQMD 24a-2	Netting	Russell Is.	1	100	96	98		3
<i>A. ustus</i> var. <i>laevis</i> (Block.) Th. & Rap.	PQMD 24a-2	Netting	Russell Is.	3	97	96	96		1
<i>A. ustus</i> var. <i>laevis</i> (Block.) Th. & Rap.	PQMD 24a-2	Netting	Russell Is.	4		82		53	2
<i>A. ustus</i> var. <i>laevis</i> (Block.) Th. & Rap.	PQMD 24a-2	Netting	Russell Is.	7		102		101	2
<i>A. ustus</i> var. <i>laevis</i> (Block.) Th. & Rap.	PQMD 24a-2	Netting	Russell Is.	7			96	101	2

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>A. versicolor</i> (Vuill.) Tira.	PQMD 4i	Shoe	Bougainville Is.	1	106	86	91		1
<i>A. versicolor</i> (Vuill.) Tita.	PQMD 17d	Tent	Espiritu Santo Is.	1			94	100	1
<i>A. versicolor</i> (Vuill.) Tira.	PQMD 26d	Canvas	Russell Is.	2	103		101	98	2
<i>A. versicolor</i> (Vuill.) Tira.	PQMD 26e	Canvas	Russell Is.	1	101		101	101	3
<i>A. versicolor</i> (Vuill.) Tira.	Fla C-18	Canteen bag	Florida	3		103	105		1
<i>A. versicolor</i> (Vuill.) Tira.	Fla C-18	Canteen bag	Florida	3			97		2
<i>A. versicolor</i> (Vuill.) Tira.	MIT 1c	Cellophane	India	4		108		106	1
<i>A. versicolor</i> (Vuill.) Tira.	MIT 1c	Cellophane	India	7		101		107	1
<i>A. Wentii</i> Weh.	PQMD 44a	Tobacco	S.W. Pacific	1	107	99	105		1
<i>A. Wentii</i> Weh.	PQMD 44a	Tobacco	S.W. Pacific	7		104	99	95	1
<i>Aspergillus</i> sp.	PQMD 62c	Corn cob pipe	Finsch., N.G.	2	102	104	100		0
<i>Aspergillus</i> sp.	PQMD 62e	Corn cob pipe	Finsch., N.G.	2	97	103	94		0
<i>Botryodiplodia Theobromae</i> Pat.	PQMD 2b	Duck	Bougainville Is.	1	102	97	97		1
<i>Botryodiplodia Theobromae</i> Pat.	PQMD 59d	Canvas	Finsch., N.G.	3	91	88	93		1
<i>Botryodiplodia Theobromae</i> Pat.	PQMD 67a	Trousers	Finsch., N.G.	7		92	73	83	3
<i>Brachysporium</i> sp.	PQMD 18c	Tent	Espiritu Santo Is.	1	54	38	42		1
<i>Brachysporium</i> sp.	PQMD 18c	Tent	Karachi, India	2	92	47	24		2
<i>Brachysporium</i> sp.	PQMD 38d	Tent	Karachi, India	2	79	73	55		3
<i>Brachysporium</i> sp.	PQMD 63b	Rope	Finsch., N.G.	2	84	76	55		0
<i>Brachysporium</i> sp.	PQMD 70g	Shoe	Finsch., N.G.	3	45	24	23		3
<i>Brachysporium</i> sp.	Fla B-57	Tent	Finsch., N.G.	3	72	59	44		4
<i>Brachysporium</i> sp.	Fla B-57	Tent	Finsch., N.G.	3	70	47	28		4

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Cephalosporium</i> sp.	Fla B-5	Tent	Florida	3	50	25	14		1
<i>Cephalosporium</i> sp.	Fla B-5	Tent	Florida	3	47	27	15		2
<i>Cephalothecium roseum</i> Corda	Fla B-50	Canvas	Florida	3	103	95	102		1
<i>Cephalothecium roseum</i> Corda	Fla B-50	Canvas	Florida	3	89	97	93		1
<i>Cephalothecium</i> sp.?	PQMD 124d	Duck	Panama	3	32	23	16		3
<i>Chaetomella</i> sp.	PQMD 40c	Canvas	Karachi, India	2	89	68	52		3
<i>Chaetomium funiculum</i> Cooke	PQMD 33c	Belt	New Guinea	1	13	0 ¹			4
<i>Chaetomium funiculum</i> Cooke	PQMD 34d	Canteen cover	New Guinea	1	11	0 ²			4
<i>Chaetomium funiculum</i> Cooke	PQMD 35e	Tent	New Guinea	2	0				4
<i>Chaetomium funiculum</i> Cooke	PQMD 36d	Tarpaulin	New Guinea	2	18	0			4
<i>Chaetomium funiculum</i> Cooke	PQMD 42a	Cap	Chabua, India	2	13	0			4
<i>Chaetomium funiculum</i> Cooke	Fla B-12	Tent	Florida	3	16	0			4
<i>Chaetomium funiculum</i> Cooke	Aust 12	Seed	Australia	3	25	13	7		3
<i>C. globosum</i> Kunze	PQMD 32b	Tent	New Guinea	1	18	0 ³			4
<i>C. globosum</i> Kunze	PQMD 38f	Tent	Karachi, India	2	16	0			4
<i>C. globosum</i> Kunze	PQMD 59h	Canvas	Finsch., N.G.	2	18	0			4
<i>C. globosum</i> Kunze	PQMD 71g	Glove	Finsch., N.G.	3	8	0			4
<i>C. globosum</i> Kunze	Fla C-12	Shower curtain	Florida	3	12	0			4
<i>C. globosum</i> Kunze	USDA 1042.4			1	13	0 ⁴			4
<i>C. globosum</i> Kunze	USDA 1042.4			3	5	0			4
<i>C. globosum</i> Kunze	USDA 1042.4			4	46	0		0	4

¹ Figure 2E.² Figure 2H.³ Figure 2B.⁴ Figure 2L.

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>C. globosum</i> Kunze (cont.)	USDA 1042.4			6	21	0			4
<i>C. globosum</i> Kunze	USDA 1042.4			7	42			0	4
<i>C. globosum</i> Kunze	USDA 1042.4			7		7	0		4
<i>C. globosum</i> Kunze	USDA 1042.4			5	42	27	10		4
<i>C. indicum</i> Corda	PQMD 46b	Rope	Ledo, India	2	26	0			4
<i>C. indicum</i> Corda	PQMD 47c	Wax paper	Ledo, India	2	26	0			4
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 17b	Tent	Espiritu Santo Is.	1		101	100		0
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 17e	Tent	Espiritu Santo Is.	1		80	67	49	3
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 17e	Tent	Espiritu Santo Is.	2	93	82	85		2
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 49a	Web belt	Ledo, India	2	78	69	52		4
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 52a	Shirt	Finsch., N.G.	2	112	103	106		1
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 55b	Leather	Finsch., N.G.	2	82	71	37		2
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 71b	Glove	Finsch., N.G.	3	87	86	74		1
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 119g	Canvas	Panama	3	94	91	95		1
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 120g	Tarpaulin	Panama	3	101	87	87		1
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 121e	Tarpaulin	Panama	3	103	102	101		1
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 121k	Tarpaulin	Panama	3	94	78	72		2

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Cladosporium herbarum</i> (Pers.) Link (cont.)	PQMD 122c	Duck	Panama	3	87	85	76		2
<i>Cladosporium herbarum</i> (Pers.) Link	PQMD 122e	Duck	Panama	3	79	71			3
<i>Cladosporium herbarum</i> (Pers.) Link	Fla B-2	Tent	Florida	3	100	94	88		1
<i>Cladosporium herbarum</i> (Pers.) Link	Fla B-22	Canteen cover	Florida	3	96	90	93		0
<i>Cladosporium herbarum</i> (Pers.) Link	Fla B-28	Cloth	Florida	3	81	56	36		4
<i>Cladosporium herbarum</i> (Pers.) Link	Fla B-28	Cloth	Florida	3	62	38	26		4
<i>Cladosporium herbarum</i> (Pers.) Link	Fla B-29	Cloth	Florida	3	80	69	55		4
<i>Cladosporium herbarum</i> (Pers.) Link	Fla B-29	Cloth	Florida	3	75	53	35		4
<i>Cladosporium herbarum</i> (Pers.) Link	Fla B-43	Canvas	Florida	3	101	96	92		1
<i>Cladosporium herbarum</i> (Pers.) Link	Fla B-68	Leggings	Florida	3	92	71	68		2
<i>Cladosporium herbarum</i> (Pers.) Link	Fla C-42	Pistol cover	Florida	3	87	82	67		3
<i>Cladosporium herbarum</i> (Pers.) Link	Fla C-44	Pistol cover	Florida	3	92	72	63		3
<i>Cladosporium herbarum</i> (Pers.) Link	Fla C-4	Web belt	Florida	3	103	102	102		1
<i>Cladosporium herbarum</i> (Pers.) Link	Fla C-21	Canteen bag	Florida	3	106	108	105		0
<i>Coniolyrium</i> sp.	PQMD 121f	Tarpaulin	Panama	3	46	34	25		4

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Cunninghamella echinulata</i> Thaxt.	PQMD 35c	Tent	New Guinea	1	107	111 ¹	107		0
<i>Curularia brachyspora</i> Boed.	Fla A-19			1		56	58	45	3
<i>Curularia brachyspora</i> Boed.	Fla B-8	Tent	Florida	3	51	20 ²	11		4
<i>Curularia brachyspora</i> Boed.	Fla B-9	Tent	Florida	3	57	30	13		4
<i>Curularia brachyspora</i> Boed.	Fla B-46	Canvas	Florida	3	38	13	5		4
<i>C. falcata</i> (Teh.) Boed.	PQMD 120h	Tarpaulin	Panama	3	88	81	57		3
<i>C. lunata</i> (Wakk.) Boed.	PQMD 10d	Canvas	Oro Bay, N.G.	2	56	27	22		4
<i>C. lunata</i> (Wakk.) Boed.	PQMD 34b	Canteen cover	New Guinea	2	52	18	0		4
<i>C. lunata</i> (Wakk.) Boed.	PQMD 35d	Tent	New Guinea	1	60	47	33		4
<i>C. lunata</i> (Wakk.) Boed.	PQMD 120L	Tarpaulin	Panama	3	47	21	15		4
<i>C. lunata</i> (Wakk.) Boed.	PQMD 120L	Tarpaulin	Panama	3	65	21	8		4
<i>C. lunata</i> (Wakk.) Boed.	PQMD 121d	Tarpaulin	Panama	3	62	37	23		4
<i>C. lunata</i> (Wakk.) Boed.	JQMD 492	Socks	Hollandia, N.G.	3	34	19	13		4
<i>Fusarium javanicum</i> Koord.	PQMD 23d	Rope	Russell Is.	1	47	40	33		3
<i>F. lateritium</i> Nees	PQMD 120d	Tarpaulin	Panama	3	51	34	31		4
<i>F. lateritium</i> Nees	PQMD 120d	Tarpaulin	Panama	7		27	15	10	1
<i>F. moniliforme</i> Sheld.	Fla B-13	Tent	Florida	3	57	44	36		4
<i>F. oxysporum</i> Schl.	PQMD 23a	Rope	Russell Is.	1	94	92	98		0
<i>F. oxysporum</i> Schl.	PQMD 23c	Rope	Russell Is.	1	54	43	40		3

¹ Figure 2K. ² Figure 1.

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>F. oxysporum</i> Schl. (cont.)									
<i>F. oxysporum</i> Schl.	PQMD 23e	Rope	Russell Is.	1	49	51	40		3
<i>F. oxysporum</i> Schl.	PQMD 23h	Rope	Russell Is.	1	54	49	38		1
<i>F. oxysporum</i> Schl.	PQMD 47e	Wax paper	Ledo, India	2	67	56	51		1
<i>F. oxysporum</i> Schl.	Fla B-61	Tent	Florida	3	39	27	19		4
<i>F. oxysporum</i> Schl.	Fla B-63	Tent	Florida	3	41	29	22		4
<i>F. oxysporum</i> Schl.	Fla C-8	Web belt	Florida	3	49	36	30		3
<i>F. roseum</i> Link	PQMD 38a	Tent	Karachi, India	2	93	77	66		2
<i>F. roseum</i> Link	PQMD 38e	Tent	Karachi, India	2	101	95	98		0
<i>F. roseum</i> Link	PQMD 38h	Tent	Karachi, India	2	103	95	90		0
<i>F. roseum</i> Link	PQMD 38g	Tent	Karachi, India	2	106	95	103		0
<i>F. roseum</i> Link	PQMD 40a	Canvas	Karachi, India	2	87	66	59		3
<i>F. roseum</i> Link	PQMD 125c	Canvas	Karachi, India	3	91	87	81		0
<i>F. roseum</i> Link	Fla A-17	Tent	Florida	1		76	53	49	4
<i>F. roseum</i> Link	Fla B-15	Tent	Florida	3	83	71	65		3
<i>F. roseum</i> Link	Fla B-24	Canteen cover	Florida	3	70	54	44		3
<i>F. Scirpi</i> var. <i>longipes</i> (W. & R.) W.	PQMD 23g	Rope	Russell Is.	1	58	53	38		2
<i>F. Scirpi</i> var. <i>longipes</i> (W. & R.) W.	PQMD 50f	Rope	Hawaii	2	101	101	102		0
<i>F. semitectum</i> Berk. & Rav.	PQMD 66b	Web strap	Finsch., N.G.	2	78	71	54		2
<i>F. semitectum</i> Berk. & Rav.	PQMD 122a	Duck	Panama	3	73	64	56		2
<i>F. semitectum</i> var. <i>maius</i> (Berk. & Lan.) Woll.	PQMD 121c	Tarpaulin	Panama	3		85	74		1
<i>F. Solani</i> (Mar.) Appel & Woll.	PQMD 21d	Tarpaulin	Espiritu Santo Is.	1	92	71	56		1

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>F. Solani</i> (Mar.) Appel & Woll. (cont.)	PQMD 21f	Tarpaulin	Espiritu Santo Is.	1	49	33	38		3
<i>F. Solani</i> (Mar.) Appel & Woll.	PQMD 119d	Canvas	Panama	3	85	71	57		1
<i>F. Solani</i> (Mar.) Appel & Woll.	PQMD 123h	Duck	Panama	3	53	35	27		1
<i>F. Solani</i> (Mar.) Appel & Woll.	PQMD 125b	Duck	Panama	3	42	30	25		1
<i>Fusarium</i> sp. (sect. elegans)	PQMD 125d	Duck	Panama	3	97	93	79		2
<i>Fusarium</i> sp.	PQMD 22a	Canvas Tent	Russell Is. Hawaii Florida	1	58	43	33		4
<i>Fusarium</i> sp.	PQMD 51d			2	98	100	99		0
<i>Fusarium</i> sp.	Fla B-14			3	87	77	75		0
<i>Ghiocladium roseum</i> Bain.	Fla B-34	Cloth	Florida	3	70	56	47		1
<i>Ghiocladium</i> sp. <i>Ghiocladium</i> sp. <i>Ghiocladium</i> sp. <i>Ghiocladium</i> sp. <i>Ghiocladium</i> sp. <i>Ghiocladium</i> sp. <i>Ghiocladium</i> sp. <i>Ghiocladium</i> sp. <i>Ghiocladium</i> sp. <i>Ghiocladium</i> sp. <i>Ghiocladium</i> sp.	PQMD 3a	Rope	Bougainville Is.	1		25	17		4
	PQMD 3a	Rope	Bougainville Is.	7			90		1
	PQMD 116b	Raincoat	Florida	3	45	17	6		4
	PQMD 170		India	8	93		81	60	2
	PQMD 316	Belt	Hollandia, N.G.	3	99	98	89		1
	Fla A-3		Florida	1		94	91		3
	Fla A-3		Florida	8		102	104	85	1
	USDA T-1			3	98		95	103	0
	USDA T-1			3	101		97		0
	42nd Chem. Co. No. 23a	String	Milne Bay, N.G.	2	103	98	90		1

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Glomastix convoluta</i> (Harz) Mason	PQMD 2c	Duck	Bougainville Is.	1		14	0		4
<i>Glomastix convoluta</i> (Harz) Mason	PQMD 2c	Duck	Bougainville Is.	1			0		4
<i>Glomastix convoluta</i> (Harz) Mason	PQMD 2c	Duck	Bougainville Is.	1			0		4
<i>Glomastix convoluta</i> (Harz) Mason	PQMD 4c	Shoe thread	Bougainville Is.	1		15	0		4
<i>Glomastix convoluta</i> (Harz) Mason	PQMD 4c	Shoe thread	Bougainville Is.	8		51	22	13	4
<i>Glomastix convoluta</i> (Harz) Mason	PQMD 4c	Shoe thread	Bougainville Is.	7	54	28	13		4
<i>Glomastix convoluta</i> (Harz) Mason	PQMD 4c	Shoe thread	Bougainville Is.	5	29	0			4
<i>Glomastix convoluta</i> (Harz) Mason	PQMD 124c	Duck	Panama	8		50	15	13	4
<i>Glomastix convoluta</i> (Harz) Mason	Fla F-108		Florida	8		50	25	21	4
<i>Glomastix convoluta</i> (Harz) Mason	Fla F-121		Florida	3	45	12	0		4
<i>Glomastix convoluta</i> (Harz) Mason	Fla F-121		Florida	8		16	9	0	4
<i>Glomastix convoluta</i> (Harz) Mason	Fla F-122		Florida	8		43	15	8	4
<i>Glomastix convoluta</i> (Harz) Mason	Fla F-177		Florida	8		61	35	12	4
<i>Glomastix convoluta</i> (Harz) Mason	MIT 16	Webbing	S.W. Pacific	3	27	9	8		4
<i>Glomastix convoluta</i> (Harz) Mason	MIT 16	Webbing	S.W. Pacific	8		31	11	7	4

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Humicola</i> sp.	PQMD 34e	Canteen cover	New Guinea	1	17	7 ¹	0		4
<i>Humicola</i> sp.	PQMD 34e	Canteen cover	New Guinea	3	25	18	4		4
<i>Humicola</i> sp.	PQMD 73d	Plastic canteen	Finsch., N.G.	3	15	3	0		4
<i>Humicola</i> sp.	PQMD 175	Human ear	Texas	3	30	23	23		4
<i>Humicola</i> sp.	PQMD 68f	Rubber coat	Finsch., N.G.	3	77	63	52		3
<i>Humicola</i> sp.	PQMD 71c	Glove	Finsch., N.G.	3	88	73	62		4
<i>Humicola</i> sp.	PQMD 71e	Glove	Finsch., N.G.	3	74	64	56		1
<i>Humicola</i> sp.	PQMD 71f	Glove	Finsch., N.G.	3	73	52	55		3
<i>Lichthemia Regnierii</i> (Luc. & Cost.) Vuill.	PQMD 45b	Shoe	Ledo, India	2	103	98	101		0
<i>Mennoniella echinata</i> Riv. (Gallow)	PQMD 1c	Canvas	Bougainville Is.	1		26	13		3
<i>Mennoniella echinata</i> Riv. (Gallow)	PQMD 1c	Canvas	Bougainville Is.	7		17	3	2	4
<i>Mennoniella echinata</i> Riv. (Gallow)	PQMD 50d	Rope	Hawaii	2	87	73	47		0
<i>Mennoniella echinata</i> Riv. (Gallow)	PQMD 50g	Rope	Hawaii	2	29	24	20		3
<i>Mennoniella echinata</i> Riv. (Gallow)	PQMD 51a	Tent	Hawaii	2	41	26	16		4
<i>Mennoniella echinata</i> Riv. (Gallow)	PQMD 51e	Tent	Hawaii	2	33	26	16		4
<i>Mennoniella echinata</i> Riv. (Gallow)	PQMD 72i	Leather	Finsch., N.G.	3	64	50	28		0
<i>Mennoniella echinata</i> Riv. (Gallow)	PQMD 82d	Haversack	Finsch., N.G.	3	48	36	32		0

¹ Figure 2I.

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Memnoniella echinata</i> Riv. (Gallow)	JQMD 177	Duck	Jeffersonville, Ind.	3	78	65	46		0
	Fla B-32	Cloth	Florida	3	66	50	38		0
	Fla B-66	Leggings	Florida	3	31	20	10		4
	Aust 86		Florida	3	59	41	25		0
<i>Melarrhizium brunneum</i> Petch	PQMD 191 ¹	Wireworms	Oregon	8	100		100	99	1
<i>M. Anisopliae</i> (Metsch.) Sorok.	PQMD 192 ²	Wireworms	Idaho	8	103		106	100	1
<i>Microsporium gypseum</i> (Bod.) Gui. & Grig.	PQMD 196	Wool fabric	Philadelphia	8	102		105	105	1
<i>Mucor fumosus</i> Naum.	MIT 3b	Gas mask cover	Mass.	3	104	99	96		0
<i>Myrothecium roridum</i> Tode ex Fr.	PQMD 201	<i>Aselepias</i> sp.	W. Africa	8	39	13	0		2
	PQMD 202	<i>Viola</i> sp.	England	8	35	8	0		2
	Fla B-11	Tent	Florida	3	0				4

¹ NRRL 1944. ² NRRL 1945.

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>M. verrucaria</i> (Alb. & Schw.) Ditm. ex Fr.	PQMD 34f	Canteen cover	New Guinea	1	17	11 ¹	7		4
<i>M. verrucaria</i> (Alb. & Schw.) Ditm. ex Fr.	PQMD 70h	Shoe	Finsch., N.G.	3	0				4
<i>M. verrucaria</i> (Alb. & Schw.) Ditm. ex Fr.	PQMD 203	Potato	Cypress	8	10	0	0		3
<i>M. verrucaria</i> (Alb. & Schw.) Ditm. ex Fr.	PQMD 204	Citrus fruits	S. Rhodesia	8	40	27	15		3
<i>M. inundatum</i> Tode ex Fr.	PQMD 206	<i>Russula adusta</i>	England	8	106		96	92	2
<i>Myrosporium</i> sp.?	PQMD 120f	Tarpaulin	Panama	3	44	16	7		4
<i>Oospora lactis</i> (Fres.) Lindau	PQMD 59f	Canvas	Finsch., N.G.	3	52	38	24		0
<i>Paecilomyces variotii</i> Bain.	PQMD 10a	Canvas	Oro Bay, N.G.	1		92	94	96	0
<i>Paecilomyces variotii</i> Bain.	PQMD 21a	Tarpaulin	Espiritu Santo Is.	2	106	108	103		0
<i>Paecilomyces variotii</i> Bain.	PQMD 42c	Cap	Chabua, India	2	108	106	103		0
<i>Paecilomyces variotii</i> Bain.	PQMD 47d	Wax paper	Ledo, India	2	105	105	97		0
<i>Paecilomyces variotii</i> Bain.	PQMD 72e	Leather	Finsch., N.G.	3	101	98	98		0
<i>Paecilomyces variotii</i> Bain.	PQMD 82f	Haversack	Finsch., N.G.	3	103	104	109		0
<i>Paecilomyces variotii</i> Bain.	PQMD 108e	Shoe	Florida	7		98	99	97	1
<i>Paecilomyces variotii</i> Bain.	PQMD 50e	Rope	Hawaii	2	108	103	109		0
<i>Paecilomyces variotii</i> Bain.	Fla B-6	Tent	Florida	3	103	102	97		0
<i>Paecilomyces variotii</i> Bain.	Fla B-6	Tent	Florida	3	108	95	100		0
<i>Penicillium Biongeianum</i> Zal.	PQMD 1a	Canvas	Bougainville Is.	1		103	104		1

¹ Figure 2J.

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Penicillium Biourgeianum</i> Zal. (cont.)	PQMD 1a	Canvas	Bougainville Is.	7		101	100	100	1
<i>P. chrysogenum</i> Thom	PQMD 51b	Tent	Hawaii	2	103	109	104		0
<i>P. chrysogenum</i> Thom	PQMD 60b	Rifle sling	Finsch., N.G.	2	106	107	105		0
<i>P. citrinum</i> series	PQMD 2a	Duck	Bougainville Is.	1		107	91		1
<i>P. citrinum</i> series	PQMD 4a	Shoe	Bougainville Is.	1		98	93		2
<i>P. citrinum</i> series	PQMD 4a	Shoe	Bougainville Is.	8		92	77	65	4
<i>P. citrinum</i> series	PQMD 4a	Shoe	Bougainville Is.	8		96	82	70	4
<i>P. citrinum</i> series	PQMD 41	Shoe lace	Bougainville Is.	1	98	96	91		2
<i>P. citrinum</i> series	PQMD 41	Shoe lace	Bougainville Is.	8		87	89	92	3
<i>P. citrinum</i> series	PQMD 4 o	Shoe	Bougainville Is.	1		94	92	87	2
<i>P. citrinum</i> series	PQMD 4 o	Shoe	Bougainville Is.	1		91	91	87	2
<i>P. citrinum</i> series	PQMD 7a	Raincoat	Bougainville Is.	1		100	96	91	2
<i>P. citrinum</i> series	PQMD 7a	Raincoat	Bougainville Is.	2		94	89	85	1
<i>P. citrinum</i> series	PQMD 7d	Raincoat	Bougainville Is.	1	98	100	98		2
<i>P. citrinum</i> series	PQMD 8a	Undershirt	Bougainville Is.	1		107	98	98	1
<i>P. citrinum</i> series	PQMD 8c	Undershirt	Bougainville Is.	1		83	89	78	2
<i>P. citrinum</i> series	PQMD 8g	Undershirt	Bougainville Is.	1		83	89	77	2
<i>P. citrinum</i> series	PQMD 10b	Canvas	Oro Bay, N.G.	1		87	92	72	2
<i>P. citrinum</i> series	PQMD 18d	Canvas	Oro Bay, N.G.	1		101	101		1
<i>P. citrinum</i> series	PQMD 36c	Tarpaulin	New Guinea	2	103	101	101		0
<i>P. citrinum</i> series	PQMD 59b	Canvas	Finsch., N.G.	2	103	98	101		0
<i>P. citrinum</i> series	PQMD 62a	Corn cob pipe	Finsch., N.G.	2	104	92	103		1
<i>P. citrinum</i> series	PQMD 69a	Canvas	Finsch., N.G.	2	102	98	97		0
<i>P. citrinum</i> series	PQMD 69b	Canvas	Finsch., N.G.	3	96	99	100		0
<i>P. citrinum</i> series	PQMD 489	Shoe	Finsch., N.G.	3	93	95	89		1
<i>P. citrinum</i> series	PQMD 489	Shoe	Hollandia, N.G.	3	103	104	99		1
<i>P. citrinum</i> series	PQMD 489	Shoe	Hollandia, N.G.	5	99		100		2

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>P. corylophyllum</i> (near)	PQMD 42b	Cap	Chabua, India	2	108	101	106		0
<i>P. cyaneum</i> (Bain. & Sart.) Biourge	PQMD 4k	Shoe	Bougainville Is.	1	103	98	85		1
<i>P. cyaneum</i> (Bain. & Sart.) Biourge	PQMD 7b	Raincoat	Bougainville Is.	1		100	93	96	0
<i>P. cyaneum</i> (Bain. & Sart.) Biourge	PQMD 7c	Raincoat	Bougainville Is.	1		101	96	98	0
<i>P. cyaneum</i> (Bain. & Sart.) Biourge	PQMD 53b	Leather	Finsch., N.G.	2	107	110	104		0
<i>P. Herquei</i> Bain & Sart.	PQMD 124a-1	Duck	Panama	3	105	101	97		0
<i>P. implicatum</i> series	PQMD 45a	Shoe	Ledo, India	2	103	106	106		0
<i>P. lilacinum</i> Thom	PQMD 4e	Shoe	Bougainville Is.	1	102	100	96		2
<i>P. luteum</i> series (non-ascosporic)	PQMD 17c	Tent	Espiritu Santo Is.	8		102	104	102	1
<i>P. luteum</i> series (non-ascosporic)	PQMD 28b	Tent	Kauai, T.H.	2	101	103	98		0
<i>P. luteum</i> series (non-ascosporic)	PQMD 74d	Leather glove	Finsch., N.G.	3	92	90	76		3
<i>P. luteum</i> series (non-ascosporic)	PQMD 74d	Leather glove	Finsch., N.G.	3	86	87	79		2
<i>P. luteum</i> series (non-ascosporic)	JQMD 396	Blanket	Hollandia, N.G.	3	65	24	14		4
<i>P. luteum</i> series (non-ascosporic)	JQMD 396	Blanket	Hollandia, N.G.	6	53		9		3

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>P. luteum</i> series (non-ascosporic) (cont.)	Fla A-24	Canteen cover	Florida	3	102	106	97		1
<i>P. luteum</i> series (non-ascosporic)	Fla B-17		Florida	3	77	34	18		4
<i>P. luteum</i> series (non-ascosporic)	USDA 1336.1			3	97	88	94		1
<i>P. luteum</i> series (non-ascosporic)	USDA 1336.1			3	96	98	90		1
<i>P. luteum</i> series (non-ascosporic)	Aust 41	Condenser label	New Guinea	3	91	32	18		4
<i>P. luteum</i> series (non-ascosporic)	Aust 82		Horne Is., _f	3	50	25	15		4
<i>P. majusculum</i> Westl.	PQMD 50a	Rope	Hawaii	2	102	98	97		0
<i>P. multicolor</i> series	PQMD 45g	Shoe Canvas	Ledo, India Florida	2	103	101	98		0
<i>P. multicolor</i> series	Fla B-40			3	92	93	85		1
<i>P. notatum</i> Westling	Fla B-1	Tent Condenser label	Florida	3	99	93	93		1
<i>P. notatum</i> Westling	Aust 40		New Guinea	3	105	102	96		0
<i>P. piscarium</i> Westling	PQMD 59a	Canvas	Finsch., N.G.	2	98	104	97		2
<i>P. purpurogenum</i> Stoll	PQMD 4n	Shoe	Bougainville Is.	1	105	92	83		2
<i>P. purpurogenum</i> Stoll	PQMD 4n	Shoe	Bougainville Is.	2	101	98	85		0
<i>P. purpurogenum</i> Stoll	PQMD 55d	Leather	Finsch., N.G.	2	103	96	97		1
<i>P. purpurogenum</i> series	PQMD 17j	Tent	Espiritu Santo Is.	1		105	105	100	1
<i>P. purpurogenum</i> series	PQMD 17j			8			102	105	0

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>P. roseo-purpureum</i> Dieck (or near)	PQMD 8a	Cotton under-shirt	Oro Bay, N.G.	2	95	103	103		0
<i>P. rugulosum</i> var. <i>atricolum</i> (Bain.) Thom	Fla B-16	Canteen cover	Florida	3	105	101	93		0
<i>P. rugulosum</i> var. <i>atricolum</i> (Bain.) Thom	Fla B-52	Tent	Florida	3	96	94	94		0
<i>P. sanguineum</i> Sopp.	Fla B-53	Tent	Florida	3	102	97	97		0
<i>P. spinulosum</i> Thom	PQMD 108b	Shoe	Florida	3	98	90	94		0
<i>P. Steckii</i> Zal.	PQMD 43a	Pliofilm	S.W. Pacific Cambridge, Mass. Cambridge, Mass.	2	106	101	108		0
<i>P. Steckii</i> Zal.	MIT 8	White cloth		3	93	104	99		1
<i>P. Steckii</i> Zal.	MIT 8	White cloth		3	96	87	91		1
<i>P. sulfureum</i> Sopp.	PQMD 52c	Shirt	Finsch., N.G.	2	107	104	112		0
<i>P. Sweickii</i> Zal. (probably near)	PQMD 17h	Tent	Espiritu Santo Is.	1	103	98	91		1
<i>P. Sweickii</i> Zal. (probably near)	PQMD 17h	Tent	Espiritu Santo Is.	8	106	100	101		1
<i>P. tardum</i> series	PQMD 4b	Shoe	Bougainville Is.	2		97	102		0
<i>P. tardum</i> series	PQMD 4b	Shoe	Bougainville Is.	7		104	103	99	0
<i>P. tardum</i> series	PQMD 24f	Netting	Russell Is.	1	103	101	103		0
<i>P. tardum</i> series	QJMD 236	Tarpaulin	Hollandia, N.G.	3	104	105	103		0
<i>P. tardum</i> series	QJMD 236	Tarpaulin	Hollandia, N.G.	6	100	100	102		0

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>P. virido-olbus</i> Sopp.	PQMD 53a	Leather	Finsch., N.G.	2	104	108	107		0
<i>P. Westlingi</i> Zal.	JQMD 238	Tarpaulin	Hollandia, N.G.	3	97	103	100		0
<i>P. Westlingi</i> Zal.	JQMD 238	Tarpaulin	Hollandia, N.G.	6	95		94		0
<i>P. Westlingi</i> Zal.	JQMD 311	Belt	Hollandia, N.G.	3	97	103	100		1
<i>P. Westlingi</i> Zal.	JQMD 311	Belt	Hollandia, N.G.	6	98		92		2
<i>P. Wortmanni</i> Klock.	PQMD 54b	Tent	Finsch., N.G.	6	97	102	96		1
<i>Penicillium</i> sp.	PQMD 4p	Tent	Finsch., N.G.	1		94	92	87	2
<i>Penicillium</i> sp.	PQMD 4p	Tent	Finsch., N.G.	7		100	101	100	1
<i>Penicillium</i> sp.	PQMD 17f	Tent	Espiritu Santo Is.	1			96	96	0
<i>Penicillium</i> sp.	PQMD 17f	Tent	Espiritu Santo Is.	8		100	104	101	0
<i>Penicillium</i> sp.	PQMD 17k	Tent	Espiritu Santo Is.	1			103	103	0
<i>Penicillium</i> sp.	PQMD 17k	Tent	Espiritu Santo Is.	8		107	99	101	1
<i>Penicillium</i> sp.	PQMD 26c	Canvas	Russell Is.	1	109		96		2
<i>Penicillium</i> sp.	PQMD 31b	Canvas	New Guinea	1	103	105	105		0
<i>Penicillium</i> sp.	PQMD 31e	Canvas	New Guinea	1	101	100	98		2
<i>Penicillium</i> sp.	PQMD 33b	Belt	New Guinea	1	100	100 ¹	98		2
<i>Penicillium</i> sp.	PQMD 34a	Canvas	New Guinea	1	105	105 ²	96		2
<i>Penicillium</i> sp.	PQMD 47b	Wax paper	Ledo, India	2	94	85	94		1
<i>Penicillium</i> sp.	PQMD 49b	Web belt	Ledo, India	2	96	92	97		1
<i>Penicillium</i> sp.	PQMD 55a	Leather	Finsch., N.G.	2	100	108	102		1
<i>Penicillium</i> sp.	PQMD 57b	Imitation leather	Finsch., N.G.	2	101	98	107		0
<i>Penicillium</i> sp.	PQMD 58a	Leatherette	Finsch., N.G.	2	108	99	103		1
<i>Penicillium</i> sp.	PQMD 58f	Leatherette	Finsch., N.G.	2	104	105	97		1

¹ Figure 2D. ² Figure 2F.

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Penicillium</i> sp. (cont.)									
<i>Penicillium</i> sp.	PQMD 62b	Corncob pipe	Finsch., N.G.	2	104	108	102		0
<i>Penicillium</i> sp.	PQMD 64d	Shirt	Finsch., N.G.	2	97	99	96		1
<i>Penicillium</i> sp.	PQMD 65a	Canvas	Finsch., N.G.	2	93	101	94		1
<i>Penicillium</i> sp.	PQMD 71a	Canvas glove	Finsch., N.G.	3	92	96	99		0
<i>Penicillium</i> sp.	PQMD 71b	Canvas glove	Finsch., N.G.	3	97	102	95		0
<i>Penicillium</i> sp.	PQMD 72b	Leather	Finsch., N.G.	3	95	101	95		0
<i>Penicillium</i> sp.	PQMD 72b	Leather	Finsch., N.G.	3	92	97	89		0
<i>Penicillium</i> sp.	PQMD 111b	Rope	Florida	3	87	66	34		0
<i>Penicillium</i> sp.	PQMD 124b	Duck	Panama	3	100	105	103		4
<i>Penicillium</i> sp.	PQMD 179	Cork gasket	Jeffersonville, Ind.	3	107	103			0
<i>Penicillium</i> sp.	Fla A-21			1					0
<i>Penicillium</i> sp.	Fla B-25	Cloth	Florida	3	100	101	94	111	1
<i>Penicillium</i> sp.	Fla B-39	Canvas	Florida	3	91	90	100		0
<i>Penicillium</i> sp.	Fla B-41	Canvas	Florida	3	84	101	88		2
<i>Penicillium</i> sp.	Fla B-64	Legging	Florida	3	94	98	96		0
<i>Penicillium</i> sp.	Fla B-54	Tent	Florida	3	82	46	27		0
<i>Penicillium</i> sp.	Fla B-65	Legging	Florida	3	100	96	99		4
<i>Penicillium</i> sp.	Fla C-5	Web belt	Florida	3	105	108	110		0
<i>Penicillium</i> sp.	Aust 114	Blood serum	Sydney, Aust.	3	107	105	103		0
<i>Pestalotia</i> sp.	Aust 8	Canvas	New Guinea	3	75	57	56		1
<i>Pestalotia</i> sp.	PQMD 2d	Duck	Bougainville Is.	1		32	10	0	3
<i>Pestalotia</i> sp.	PQMD 2d	Duck	Bougainville Is.	7		31	7	4	4
<i>Pestalotia</i> sp.	PQMD 119a	Canvas	Panama	3	85	75	45		2
<i>Pestalotia</i> sp.	PQMD 119b	Canvas	Panama	3	80	70	56		3
<i>Pestalotia</i> sp.	PQMD 119b	Canvas	Panama	8		38	17	9	4
<i>Pestalotia</i> sp.	PQMD 120b	Tarpaulin	Panama	3	79	69	65		1

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Pestalotia</i> sp. (cont.)									
<i>Pestalotia</i> sp.	PQMD 121b	Tarpaulin	Panama	3	104	100	103		2
<i>Pestalotia</i> sp.	PQMD 121L	Tarpaulin	Panama	3	86	80	70		3
<i>Pestalotia</i> sp.	PQMD 121L	Tarpaulin	Panama	8		81	50	37	4
<i>Pestalotia</i> sp.	PQMD 125a	Duck	Panama	3	84	84	82		1
<i>Pestalotia</i> sp.	Fla C-72	Cord	Florida	3	86	70	67		1
<i>Phoma</i> sp.	PQMD 13e	Canvas	Espiritu Santo Is.	1	77	65	67		1
<i>Phoma</i> sp.	PQMD 48c	Tent	Ledo, India	2	63	54	35		2
<i>Phoma</i> sp.	PQMD 120k	Tarpaulin	Panama	3	82	67	39		3
<i>Phoma</i> sp.	PQMD 120k	Tarpaulin	Panama	8	63	31	9		3
<i>Phoma</i> sp.	PQMD 122f	Duck	Panama	3	75	64	54		1
<i>Phoma</i> sp.	PQMD 121h	Tarpaulin	Panama	3	64	30	20		3
<i>Phoma</i> sp.	Fla B-30	Cloth	Florida	3	52	26	18		2
<i>Pullularia pullulans</i> (D By.) Berkth.	PQMD 72h	Leather	Finsch., N.G.	3	96	94	97		0
<i>Pullularia pullulans</i> (D By.) Berkth.	PQMD 72h	Leather	Finsch., N.G.	3	92	98			0
<i>Pullularia pullulans</i> (D By.) Berkth.	PQMD 72h	Leather	Finsch., N.G.	8	100	98	104		1
<i>Pullularia pullulans</i> (D By.) Berkth.	PQMD 122b	Duck	Finsch., N.G.	3	95	95	98		0
<i>Pullularia pullulans</i> (D By.) Berkth.	JQMD 364	Belt	Hollandia, N.G.	3	102	98	90		0
<i>Pullularia pullulans</i> (D By.) Berkth.	Fla B-37		Florida	3		107	106		0
<i>Pullularia pullulans</i> (D By.) Berkth.	Fla F-44		Florida	3	103	105	93		0

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Pyrenochaeta</i> sp.	PQMD 29b	Tarpaulin	Kauai, T.H.	2	51	27	22		4
<i>Rhizopus arrhizus</i> Fisch.	PQMD 46c	Rope	Ledo, India	2	101	103	103		0
<i>Sarcopodium fuscum</i> (Corda) Sacc.	PQMD 28a	Tent	Kauai, T.H.	1	46	32	14		4
<i>Sarcopodium fuscum</i> (Corda) Sacc.	PQMD 28a	Tent	Kauai, T.H.	3	25	11	5		0
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bain.	Fla C-28	String	Florida	3	100	103	92		1
<i>Septomyxa affinis</i> (Sherb.) Woll.	PQMD 40b	Tent	Karachi, India	2	106	101	87		0
<i>Sphaeropsis</i> sp.	PQMD 46h	Rope	Ledo, India	2	103	95	93		2
<i>Sphaeropsis</i> sp.	PQMD 46h	Rope	Ledo, India	3	98	101	96		2
<i>Sporotrichum</i> sp.	PQMD 4f	Shoe	Bougainville Is.	1	100	92	89		0
<i>Stachybotrys atra</i> Corda	Fla B-10	Tent	Florida	3	18	19	19		4
<i>Stachybotrys atra</i> Corda	Fla B-31	Cloth	Florida	3	23	20	16		4
<i>Stachybotrys atra</i> Corda	Fla B-62	Tent	Florida	3	56	41	31		2
<i>Stachybotrys</i> sp.	Fla C-10	Shower curtain	Florida	3	0				4
<i>Stemphylium consortiale</i> (Thüm) Gr. & Sk.	PQMD 41b	Rope	Karachi, India	2	66	48	32		4

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Syncephalastrum racemosum</i> (Cohn) Schr.	PQMD 57a	Imitation leather	Finsch., N.G.	2	106	102	102		1
<i>Syncephalastrum racemosum</i> (Cohn) Schr.	Fla B-23	Canteen cover	Florida	3	98	97	99		1
<i>Syncephalastrum racemosum</i> (Cohn) Schr.	Fla B-35	Cloth	Florida	3	95	96	100		1
<i>Thielavia Sepedonium</i> Emm.	PQMD 46a	Rope	Ledo, India	2	32	16	0		4
<i>Thielavia Sepedonium</i> Emm.	PQMD 47g	Wax paper	Ledo, India	2	15	0			4
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 6a	Tent	Bougainville Is.	1	18	10	8		4
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 66a	Strap	Finsch., N.G.	2	29	18	7		4
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 13b	Canvas	Espiritu Santo Is.	1		91	101	98	0
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 18a	Tent	Espiritu Santo Is.	1	72	36	13		2
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 18a	Tent	Espiritu Santo Is.	3		78	71	71	4
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 18e	Tent	Espiritu Santo Is.	1	47	19	15		4
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 21b	Tarpaulin	Espiritu Santo Is.	1	60	31	16		4
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 22d	Tarpaulin	Russell Is.	1	36	25	16		4
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 22e	Canvas	Russell Is.	1	53	33	18		3

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Trichoderma viride</i> (Pers.) Harz (cont.)	PQMD 33a	Canvas	New Guinea	1	91	72 ¹	58		4
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 46d	Rope	Ledo, India	2	56	29	16		4
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 63d	Cotton rope	Finsch., N.G.	2	100	99	98		0
<i>Trichoderma viride</i> (Pers.) Harz	PQMD 120o	Tarpaulin	Panama	3	98	94	44		3
<i>Trichoderma viride</i> (Pers.) Harz	JQMD 314	Belt	Hollandia, N.G.	3	86	65	31		3
<i>Trichoderma viride</i> (Pers.) Harz	JQMD 315	Belt	Hollandia, N.G.	3	56	26	28		3
<i>Trichoderma viride</i> (Pers.) Harz	JQMD 474	Raincoat	Hollandia, N.G.	3	67	27	20		4
<i>Trichoderma viride</i> (Pers.) Harz	Fla A-4		Florida	1	47	19	15		4
<i>Trichoderma viride</i> (Pers.) Harz	Fla B-3	Tent	Florida	3	101	55	22		4
<i>Trichoderma viride</i> (Pers.) Harz	Fla C-55	Socks	Florida	3	31	16	7		4
<i>Trichophyton</i> sp.?	PQMD 199	Wool fabric	Philadelphia	8	105		101	104	1
<i>Trichophyton</i> sp.?	PQMD 208	Wool fabric	Philadelphia	8	106		99	106	1
<i>Trifarctium dependens</i> Limb. & Asperg. versicolor (Vuill.) Tir.	PQMD 4g	Shoe	Bougainville Is.	1	105	101	101		1

¹ Figure 2C.

TABLE II—continued

Species	Culture Number	Source	Locality	Method Variant	Per Cent Strength Retained				Growth at End of Experiment
					6 Days	9 Days	12 Days	15 Days	
<i>Tritirachium</i> sp.	PQMD 164	Veg. ivory buttons	Tropics	8	103		100	101	1
<i>Verticillium</i> sp.?	Fla B-51	Canvas	Florida	3	54	32	27		3
<i>Verticillium</i> sp.?	Fla B-59	Tent	Florida	3	51	25	15		2
<i>Verticillium</i> sp.?	Fla C-7	Web belt	Florida	3	70	41	29		0
<i>Verticillium</i> sp.?	PQMD 125e	Duck	Panama	3	27	23	16		1
<i>Zygorhynchus Moelleri</i> Vuill. with internal parasite	Fla A-29		Florida	3	36	14	7		2
{ Undet. Dematiaceae Undet. Dematiaceae Undet. Dematiaceae	PQMD 14c	Rope	Espiritu Santo Is.	1					0
	PQMD 14c	Rope	Espiritu Santo Is.	7	94	103	100	99	1
	PQMD 69c	Coat	Finsch., N.G.	3	96	94	100		1
Undet. Moniliaceae	PQMD 119c	Canvas	Panama	3	91	71	62		2

The figures cited in the right hand column to indicate the estimated amount of growth at the end of the incubation period are as follows: (0) meaning no growth visible to the naked eye (FIG. 2 A, K); (1) a small amount of growth in the form of a scarcely visible fuzziness which might be expected simply as a result of spore germination and the utilization of a small amount of food material available as impurities (FIG. 2D); (2) slightly more growth, often accompanied by sparse or inhibited fruiting and not necessarily an indication of cellulose utilization (FIG. 2F, G); (3) well developed fungus growth which may always be taken as indicative of cellulolytic activity (FIG. 2C); and (4) meaning that the fabric is covered or nearly so with a well developed growth typically indicative of strong activity (FIG. 1; 2B, E, H, I, J, L; 3).

SUMMARY AND COMMENT

Results are reported of a series of assays to determine roughly the cellulolytic activity, as measured by decline in tensile strength of cotton fabric, of certain fungi isolated from textiles and related materials deteriorated in the tropics. The total number of assays was 584. These represent 453 cultures distributed among 45 genera. Of the 453 cultures, 310 are identified to series, species or variety and 143 are determined only to genus or section of genus. The total of 310 cultures determined beyond generic rank, *i.e.*, the number of species or approximate species, is 91. The great majority are the imperfect stages of Ascomycetes, *i.e.*, Fungi imperfecti; a few are perithecial and a few are Mucorales. No Basidiomycetes are included. Cellulolytic activity is indicated for 41 of the determined species whereas 50 gave negative results.

The following summarizing comments are offered:

1. *Mucorales*. Six species were tested: *Absidia capillata*, *Cunninghamella echinulata*, *Lichthemia Regnieri*, *Mucor fumosus*, *Rhizopus arrhizus*, and *Syncephalastrum racemosum*. In all cases the results were negative. A seventh culture, *Zygorhynchus Moelleri* (Fla A-29), presented an interesting case. Continued investigation of its apparent cellulolytic activity finally demonstrated this to be due to the action of an internal Dematiaceous parasite, which, following inoculation of the cloth, grew out of its host and

attacked the fabric. A few Mucorales have been tested previously (1, 2, 3, 9, 13, 16), and the results always have been negative. Evidence is accumulating that the Mucorales lack the ability to utilize cellulose as a source of carbon or to degrade it directly.

2. *Aspergillus*. A total of 125 cultures were tested. These represent all the 14 groups of Thom and Raper (15) except the *A. clavatus* and *A. candida* groups. Of the remaining 12 groups, cellulolytic activity was demonstrated in five. The results are summarized briefly below by group.

a. *The A. glaucus group*. Five cultures representing *A. Chevalieri* and its variety *intermedius* and four of *A. repens* were assayed. The results were in all cases clearly negative. This is in agreement with results obtained by Galloway (2), although such forms are sometimes recorded (2, 13) as occurring on cotton goods.

b. *The A. fumigatus group*. The one culture of *A. Fisheri* tested gave positive results in each of three assays, whereas three strains of *A. fumigatus* were positive and three negative. The results obtained here, together with those recorded previously (6, 9, 13, 16), indicate that capacity to decompose cellulose probably is general in the group.

c. *The A. nidulans group*. Seven strains of *A. unguis* gave clearly negative results. The one culture of *A. nidulans* tested gave doubtful results in one case and negative in a somewhat modified repeat assay. Previous tests (2, 9, 13) of what has been recorded as *A. nidulans* have given contradictory results.

d. *The A. ustus group*. Cultures of *A. ustus* and its variety *laevis* behaved rather peculiarly in that they made considerable growth without causing a pronounced decline in tensile strength. It appears possible, or even probable, that cellulolytic ability will be found to be general in the group under a relatively restricted optimal set of conditions.

e. *The A. flavipes group*. Weak cellulolytic activity was demonstrated for all three of the cultures of *A. flavipes* tested. This is in conformity with the results of later more detailed work (unpublished) in connection with the present program. Galloway (2) recorded its action as strong based on visual growth on filter paper.

f. *The A. versicolor group.* The numerous cultures of *A. versicolor* and *A. Sydowi* were all negative and give strong indication that members of the group are unable to attack cellulose despite previous literature records (2, 9) to the contrary.

g. *The A. terreus group.* The seven cultures of *A. terreus* which were tested grew and fruited readily and consistently on test strips. This perhaps is the most strongly and consistently cellulolytic species of the genus. Other workers (2, 9) have obtained similar results.

h. *The A. niger group.* This has been a very controversial group in deterioration laboratories with several early literature records (13) claiming cellulolytic properties for its members, partly on circumstantial evidence. Of the numerous cultures here tested, positive results were obtained for only one, and possibly a second, both of which were later determined as *A. luchuensis*. No effort has been made to identify the other cultures beyond the group category, except to ascertain that there were no additional cultures of *A. luchuensis* among them. A more detailed study of cellulolytic activity in the *A. niger* group is in progress.

i. *A. Wentii group.* The single culture tested gave negative results on both bleached sheeting and gray duck. Others (2, 13) have indicated positive results.

j. *The A. Tamarii group.* No activity could be demonstrated in three isolates determined as *A. Tamarii*. Galloway (2) recorded moderate growth on filter paper and Furry & Zametkin (1) claimed decline in strength of cotton duck.

k. *The A. flavus-Oryzae group.* No cellulolytic activity was demonstrated for the numerous cultures identified as *A. flavus* and *A. Oryzae*. This conflicts with results obtained by others (1, 2, 13).

l. *The A. ochraceus group.* Results were negative for the two cultures identified as *A. ochraceus*. Galloway (2) reported moderate visual growth on filter paper.

3. *Penicillium.* A total of 126 assays were conducted, including 94 cultures of which 62 are determined approximately to species and 32 remain unidentified. The only clearly positive results obtained were for certain, or most, of the 9 strains of *P. luteum*

that were tested, for some strains of the *P. citrinum* series and for two unidentified forms, i.e., PQMD 111b and Fla B-54. *P. citrinum* is isolated extremely commonly from fabrics and similar materials exposed in the tropics and should be given further attention. In the review of Thaysen and Bunker (13) of twenty years ago 19 species of *Penicillium* were recorded as having given positive results based mostly on visual growth on filter paper or on the clearing of a cellulose agar medium. Of the nineteen species recorded there as cellulolytic, six appear in the table of this paper. The results correlate for only one species, *P. luteum*. The positive results previously recorded for *P. chrysogenum*, *P. notatum*, *P. purpurogenum*, *P. rugulosum*, and *P. spinulosum* could not be confirmed in the present study. Taxonomic confusion in such a difficult group could account for this, at least in part. Since the appearance of the book by Thaysen and Bunker only scanty mention has been made of members of the genus relative to cellulose decomposition (1, 2, 3, 9).

4. *Moniliaceae*.

a. *Fusarium*. The results of tests of 34 isolates representing 10 species and varieties indicate that substantial cellulolytic activity probably is the rule in this genus. This is in agreement with the several scattered literature records (1, 2, 9, 13) of tests of undetermined species. The results indicated in the accompanying table are not to be considered significant to the extent that they may be considered as representing species differences with respect to cellulolytic ability.

b. *Gliocladium*. The eight cultures listed under *Gliocladium* sp. represent a single species. It is the species represented by Weindling's culture G-1, which, together with one or two other strains, has been used in the production of gliotoxin and viridin. It has generally been referred to as *Gliocladium fimbriatum* or *Trichoderma viride*, but is distinct from either of these. This question is being taken up in another paper. Cellulolytic activity appeared to be strong in some strains and weak or absent in others and some evidence of instability in this respect has been noted. One strain of *G. roseum* was tested and proved to be cellulolytic.

c. *Humicola*. The 7 cultures tested, representing 2 species, all were found to be strongly cellulolytic. They are considered un-

identifiable, pending the appearance of a better basic taxonomy of the genus.

d. Trichoderma viride. Of seventeen strains tested, all proved to be relatively strongly cellulolytic. The species is isolated very frequently from fabrics and is of common and universal occurrence throughout the tropic and temperate parts of the world. It is fairly generally known as a cellulose destroyer, and is considered to be active in the soil in this respect.

e. Tritirachium. *T. dependens* and an undetermined species were clearly negative.

f. Other Moniliaceae. Results for members of several other less prominent genera for which space cannot be taken for detailed summarization, may be found by turning the pages of the table. Genera represented are: *Acladium*, *Acremonium*, *Cephalosporium*, *Microsporum*, *Oospora*, *Paecilomyces*, *Sarcopodium*, *Scopulariopsis*, *Septomyxa*, *Sporotrichum*, and *Verticillium*. Occasional mention of members of some of these genera will be found in papers listed in the bibliography. Some are in genera where specific identification is impossible, and even where the possibility of a really meaningful generic disposition is extremely doubtful.

5. *Dematiaceae.*

a. Alternaria. Five cultures were tested, all of which were identified with strains commonly referred to *A. tenuis*. Relatively uniform and fairly strong activity is indicated. Previously recorded tests of unidentified species (4, 9, 13) have indicated similar results.

b. Brachysporium. Six cultures were tested without any attempt having been made to match them or to identify any of them to species. Activity similar to that of the more or less related *Alternaria* group is indicated.

c. Cladosporium herbarum. Of universal occurrence on fabrics and other materials exposed in nature, it is in need of further attention both taxonomically and physiologically. The results for the many cultures tested here and from various literature records (4, 9, 13) indicate varying, and, on the whole, not strong cellulolytic activity for the forms commonly loosely placed here.

d. Curvularia. Ten isolates distributed among three species gave results similar to those obtained for *Alternaria* and *Brachy-*

sporum. Within the accuracy of these tests no cellulolytic differences are noted among the species.

e. *Gliomastix convoluta*. Eight cultures tested were all strongly cellulolytic. Culture 4c was selected for detailed nutritional studies in a Quartermaster sponsored project, but the results are not as yet published. The identification is uncertain.

f. *Memnoniella echinata*. All of a total of eleven cultures tested were strongly cellulolytic.

The figures in the table are interesting in several respects. Since little mycelial growth is made on a cellulose substratum the visual growth rating refers largely to the amount of fruiting. In all instances the fabric was either covered at the end of twelve days with black sporulation or there was no fruiting at all. Intermediates did not occur. The contrast among strains in this respect was more striking than among strains of any other species assayed. Cellulolytic activity appeared to be correlated positively with fruiting even though it was fairly strong where no fruiting occurred. Interpreting the table in the light of the work of Marsh & Bollenbacher (7), who demonstrated the need for an external source of biotin in all of six cultures of the species which they tested, one might postulate that both biotin requiring and non-biotin requiring strains exist. Strain Aust 86 in the table was among those shown (l.c.) to require biotin. PQMD 1c would probably be supplied a sufficient amount by the test method used. Under the more highly purified method variants 2 and 3, by which the other strains were tested, biotin should be absent or nearly so.

6. *Tuberculariaceae*. Represented in these tests by three species of *Myrothecium* of which *M. verrucaria* (Syn.: *Metarrhizium glutinosum* Pope) and the very closely allied *M. roridum* are among the strongest cellulose decomposers yet found. (Cf. also 4, 10, 18.) A culture of *M. inundatum* exhibited weak or negative action. Detailed nutritional studies are in progress as a part of the present program.

7. *Stilbaceae*. Represented only by a culture identified as *Arthrosporium* sp., which evidenced no activity.

8. *Sphaeropsidales*.

a. *Botryodiplodia Theobromae*. The results obtained for three cultures which were tested indicated negative, or at most, very weak activity. This is a common tropical species capable of invading the living tissues of a wide variety of plants. It is isolated from fabrics very frequently but there is as yet no evidence that it is there other than as spores deposited from the atmosphere. It should be given further attention.

b. *Coniothyrium*, *Phoma*, and *Pyrenochaeta*. A complex of pycnidial forms having very small ellipsoid spores and referable to these genera are found producing pycnidia on fabrics with considerable frequency, often accompanied by staining. Numerous cultures are at hand and those tested proved to be strongly cellulolytic.

9. *Ascomycetes*

a. *Chaetomium*. Fifteen cultures were tested. These included seven of *C. funiculum*, six of *C. globosum*, and two of *C. indicum*. Since *C. globosum* has long been known as a strong cellulose-destroyer (11, 13, 14) and since a wide range of additional species were tested recently (5), the results presented here are not of great interest. They do, however, suggest that within limits there is no great variation in different strains of the three species, and that a given strain of any one of the three species is likely to be about as strong cellulolytically as the standard strain, USDA 1042.4, used in assessment and procurement testing of cotton fabrics.

b. *Thielavia Sepedonium*. The two cultures tested exhibited action of the same order of magnitude as that of species of *Chaetomium*.

ACKNOWLEDGMENTS

Professor William H. Weston, Jr., of Harvard University, then in the capacity of Consultant to the Office of the Quartermaster General, was charged with the responsibility for the organization of this laboratory and the direction of the early phases of its research program. The studies reported in this paper were conducted during Professor Weston's active association with the laboratory. The writers feel deeply indebted to him, not only for his research guidance, but also for his personal encouragement and

inspiration. The assistance of the following individuals in the identification of cultures within their particular fields of interest is gratefully acknowledged: Dr. Lawrence Ames, Dr. Victor M. Cutter, Dr. W. H. Diehl, Dr. J. Walton Groves, Dr. D. H. Linder, Dr. E. W. Mason, Dr. Kenneth B. Raper, Prof. C. D. Sherbakoff, and Prof. William C. Snyder.

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EXPLANATION OF FIGURES

FIG. 1. Cloth test strips incubated nine days under method variant 3 of Table I. (Magnification $\times 1$.) At left uninoculated control, at right inoculated with *Curvularia brachyspora*, Fla B-8.

FIG. 2. Cloth test strips inoculated with various fungi and incubated nine days under method variant 1 of Table I. (Magnification $\times 1$.) A, Control, incubated without having been inoculated; B, *Chaetomium globosum*, PQMD 32b; C, *Trichoderma viride*, PQMD 33a; D, *Penicillium* sp., PQMD 33b; E, *Chaetomium funicolum*, PQMD 33c; F, *Penicillium* sp., PQMD 34a; G, *Aspergillus niger*, PQMD 34c; H, *Chaetomium funicolum*, PQMD 34d; I, *Humicola* sp., PQMD 34e; J, *Myrothecium verrucaria*, PQMD 34f; K, *Cunninghamella echinulata*, PQMD 35c; L, *Chaetomium globosum*, USDA 1042.4. The spotting of strips A, D, F, and K is due to uneven contact with the plate glass on which they were placed for photographing immediately after removal from the incubation vessels; little or no fungous growth is visible in the photograph.

FIG. 3. Strips of gray cotton duck incubated twelve days under method variant 5 of Table I. (Magnification $\times 1$.) At left uninoculated control, at right inoculated with *Aspergillus luchuensis*.

THREE ZOÖPAGACEAE THAT SUBSIST BY CAPTURING SOIL AMOEBAE

CHARLES DRECHSLER¹

(WITH 4 FIGURES)

As the unusually cool humid weather that prevailed during the summer of 1946 in Maryland, Virginia, and North Carolina furnished conditions of temperature and moisture under which the fungi destructive to eelworms and rhizopods have been found developing most abundantly in the laboratory, it was expected that decaying plant materials collected in later months might yield many zoöpagaceous forms not ordinarily encountered. However, when vegetable detritus taken from woods, fields, and gardens, in various localities within the states mentioned, was planted on Petri plates of maizemeal agar suitably permeated with *Pythium* mycelium, the resulting cultures were in most instances ruined through an overwhelming development of plasmodia belonging to different species of Myxomycetes. Usually these plasmodia suppressed multiplication of rhizopods and eelworms at an early stage, and thereby forestalled virtually all development of fungi habitually subsisting on these animals. In other instances zoöpagaceous fungi became established and began to sporulate in noticeable quantity, but would then be obliterated under shifting streams of slime. In a few cultures, fortunately, plasmodia failed to develop; and here abundant multiplication of rhizopods and eelworms took place, together with development of fungi destructive to them. Among these fungi were observed the three predaceous forms herein described as new members of the Zoöpagaceae.

Trouble from plasmodia of Myxomycetes has been experienced now and then in earlier years, though not on a serious scale. Or-

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dinarily cultures prepared with decaying plant materials collected outdoors in Maryland and Virginia are less likely to be ruined by slime molds than from infestation with mites, annelids, tardigrades, or rotifers. Mites have been found especially troublesome in cultures planted with decaying material taken from greenhouses, or with decaying plant detritus collected outdoors in such warm states as Florida and Louisiana. While these pests do not necessarily prevent vegetative development of nematode-destroying and rhizopod-destroying fungi deep under the surface of an agar culture, they usually grind up the surface layer so badly by their manner of feeding that the reproductive apparatus of all except the most robust Zoöpagaceae is broken up beyond all hope of recognition, and often the conidial apparatus of the sturdiest forms is mangled so severely as to render it unfit for study. The use of herbarium poisons, such as naphthalene and paradichlorobenzene, in mite-infested cultures has not been found advantageous since rhizopods and nematodes succumb quickly in the presence of these volatile compounds. Storage of the cultures at low temperatures similarly entails some disadvantage, for thereby not only the infesting mites but also the microscopic animals and associated fungi similarly preferring warm conditions—the very forms, indeed, that could be expected to be most characteristic of mite-infested material—are largely if not wholly suppressed.

The minute annelids that frequently appear in Petri plate cultures five to ten days after decaying material has been added cause even more serious damage than mites, since they burrow through the agar at all depths, creating havoc in their paths. These unwanted animals seem to hatch most quickly when a rather soft agar medium is employed, or when some free liquid water collects around the material planted, yet may become troublesome even when firm agar is employed and when little moisture condenses. Difficulty with them may be largely obviated, as a rule, by taking care in collecting leaf mold or other decaying plant detritus to include no considerable admixture of sand, clay, loam, or gravel from the underlying soil. Destruction by annelids is exceedingly frequent in cultures planted with ordinary soil from gardens and fields. Despite the manifestly thoroughgoing distribution of the Zoöpagaceae on all arable land, the difficulties attending annelid

infestation have strongly discouraged the use of firm soil in preparing cultures intended for the study of these fungi.

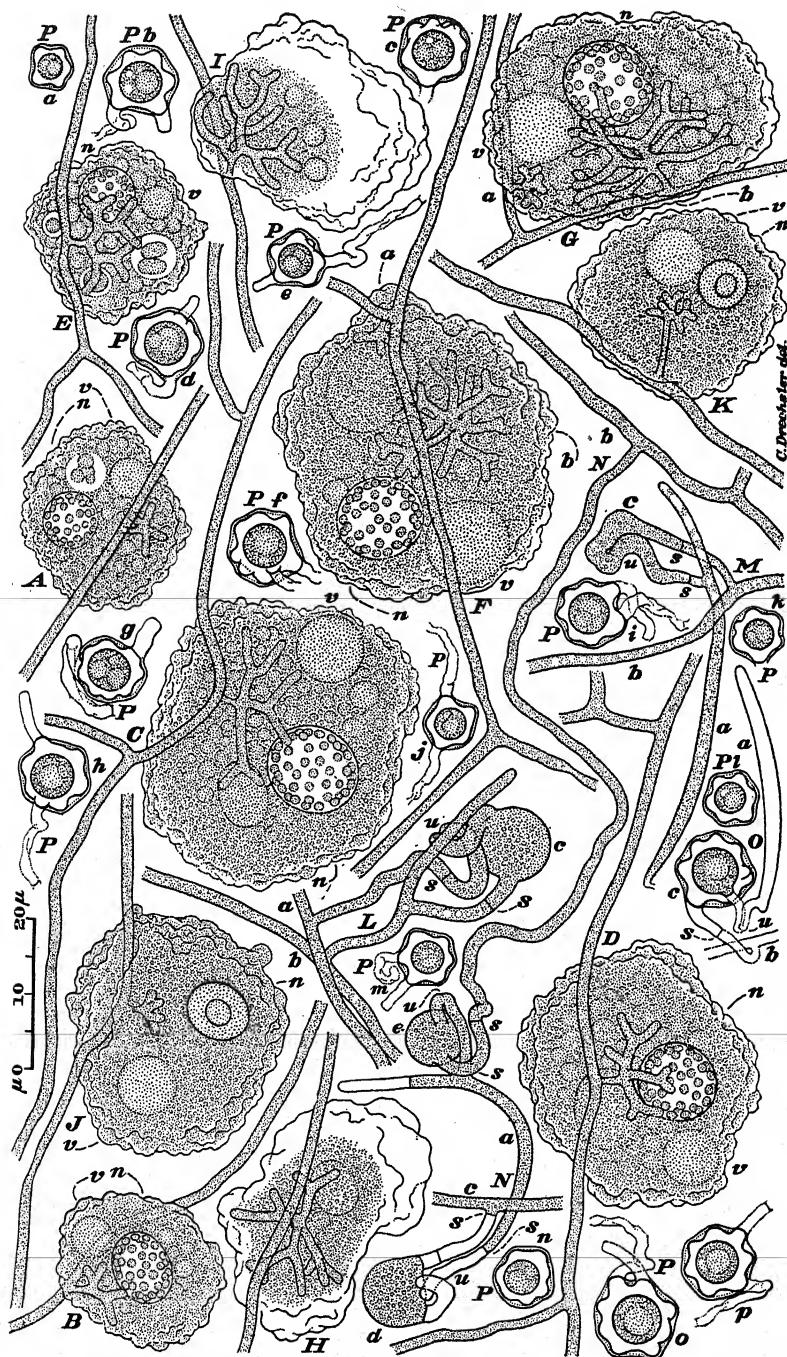
ANOTHER ACAULOPAGE WITH LONG SLENDER ROD-LIKE CONIDIA

Several maize meal-agar plate cultures that when completely overgrown with mycelium of *Pythium ultimum* Trow had been further planted through addition of decaying detritus taken on August 20, 1946, from an old pile of weeds in a field near Mayo, Maryland, were found after twenty-seven days to contain a sparse branching mycelium composed of narrow colorless aseptate hyphae (FIG. 1, A-G) to which were attached many individual *Amoebae* generally similar to one another. The captives commonly measured from 20 to 40 μ in diameter when drawn into a moderately rounded shape. They were surrounded individually by a thin yet distinctly visible pellicle, which, except in regions where pseudopodia were being extended, was handsomely disposed in delicately rippled folds. Their protoplasm, though colorless and for the most part of only moderately densely granular texture, was reduced in transparency by numerous vacuole-like inclusions of globular or ellipsoidal shape. These inclusions were mostly less than 5 μ wide, but in some animals (FIG. 1, C, D, E) one or two among them measured about 10 μ in their greatest dimension and thus rivaled the contractile vacuole (FIG. 1, A-G: v) in size. Some animals contained, besides, one or more commonplace digestive vacuoles (FIG. 1, A, E). While the single nucleus (FIG. 1, A-G: n) appeared to be generally of prolate ellipsoidal shape, the difference between its length and its width was often relatively small. In comparison with the size of the animal the nucleus seemed unusually large, its length varying commonly from 8 to 12.5 μ , and its width from 7 to 11 μ . It showed, distributed in peripheral positions, some 35 to 50 globose or slightly flattened bodies about 1 μ or slightly less in width. Among the *Amoebae* previously reported as being destroyed by members of the Zoöpagaceae the animal here concerned seemed to resemble most the species found habitually captured by my *Stylopaga rhabdospora* (5: 374-377) and my *S. cephalote* (7: 144-148). However the obscurely globuliferous structure of the protoplasm, together with

the apparently larger number and smaller size of the chromatin bodies, gives reason for presuming that the animal is specifically distinct not only from the one earlier observed serving as prey for the two species of *Stylopage*, but also from the one found parasitized by my *Cochlonema euryblastum* (10: 283-289). In the *Amoeba* parasitized by *C. euryblastum*, besides, the nucleus appeared appreciably smaller relative to the entire animal than in the *Amoeba* of globuliferous protoplasmic texture.

Following narrow perforation of its integument each captured *Amoeba* was soon invaded by a haustorium (FIG. 1, A-E), or at times by two haustoria (FIG. 1, F, a, b; G, a, b). As in *Stylopage rhabdospora* the absorptive organ here was of the familiar pedicellate type. Ramification of the assimilative elements was mainly dichotomous, though irregularity of branching appeared rather often, especially in the more elaborately developed haustoria. The number of terminal branches occasionally exceeded sixteen (FIG. 1, F, b; G, b). The assimilative elements appeared generally to be of about the same width as mycelial hyphae. Owing to the globuliferous character of the animal's protoplasm the haustorium was only somewhat indistinctly visible during the earlier stage of its development, but after most of the protoplasm had been assimilated it emerged more clearly into view (FIG. 1, H, I). Later, when the *Amoeba* was depleted of its digestible substance, the contents of the haustorium were withdrawn backward into the parent hypha; the empty tubular envelope thereupon soon fading from sight together with the empty pellicle loosely surrounding it.

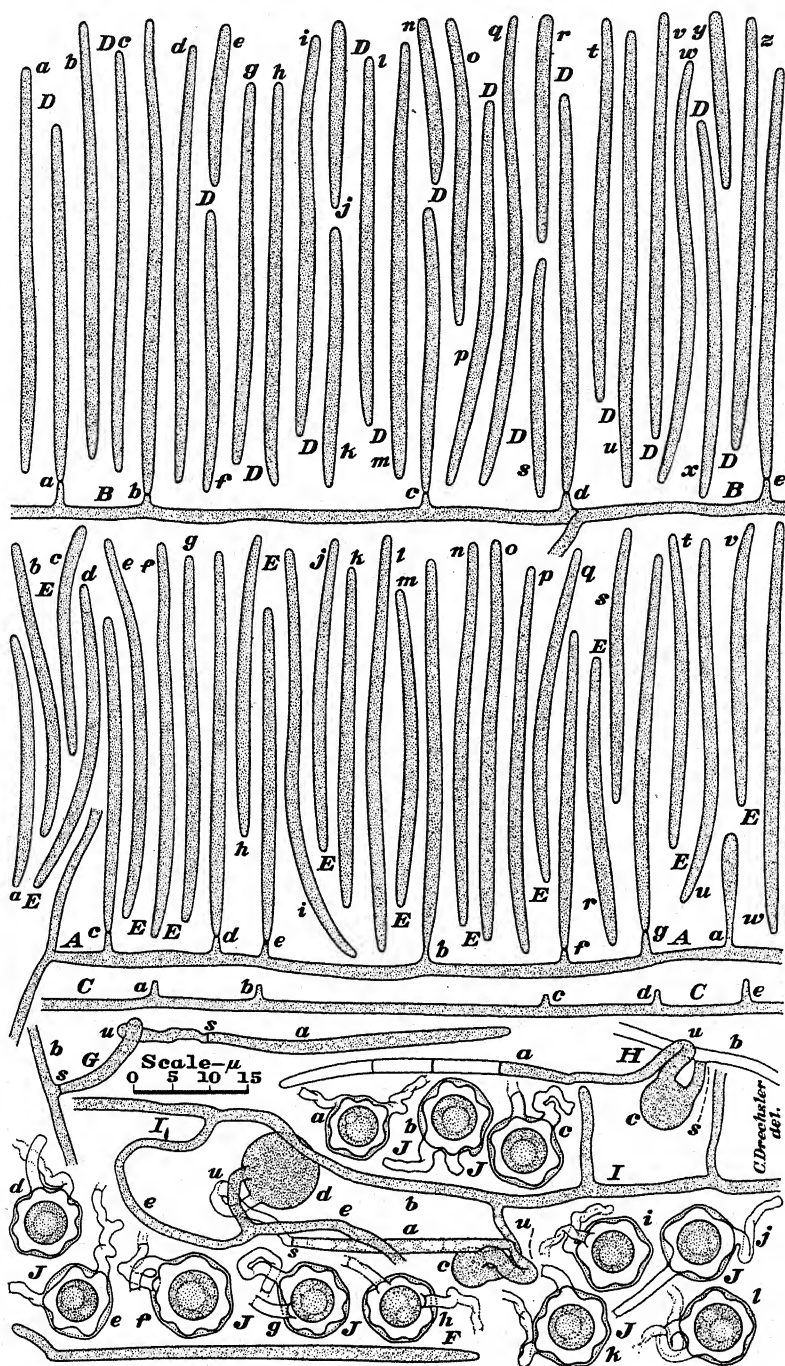
In one of the plate cultures an area occupied by mycelium of the fungus showed a few captured *Amoebae* (FIG. 1, J, K) which, though of about the same size as those taken in large numbers, were distinguished by protoplasm of commonplace granular texture and by a smaller nucleus (FIG. 1, J, n; K, n) that contained a single central endosome instead of many peripheral bodies. Despite careful scrutiny it was not possible to trace any mycelial connection between the hyphae holding fast these few animals of a different species and the hyphae holding fast the much more numerous captives. The fungus represented in these latter hyphae, therefore, cannot yet be reckoned among the small number of zoö-

FIG. 1. *Acaulopage baculispora*.

pagaceous forms definitely known to subsist on two or more distinct species of rhizopods.

The fungus gave rise abundantly to both asexual and sexual reproductive apparatus. In initiating asexual reproduction a prostrate mycelial filament would at mostly rather short intervals put forth erect narrow processes that in their upward growth widened out until they had attained a height of about $15\ \mu$ (FIG. 2, *A, a*). Thereupon they continued elongating either at a uniform width, or at a slightly diminishing width, until on attaining a definitive length of 25 to $65\ \mu$ (FIG. 2, *A, b*) a cross-wall was formed a few microns above the base to delimit, in each instance, a rod-shaped or somewhat filamentous erect conidium from the short, often slightly tapering sterigma below (FIG. 2, *A, c-g*; *B, a-e*). Collectively the conidia in an undisturbed sporulating tract offered a handsome bristling display. They readily became disjointed, however, when they were jostled by roving eelworms or rotifers. The procumbent mycelial hyphae, including those beset with numerous denuded sterigmata (FIG. 2, *C, a-e*), came thereby to be partly obscured amid a disorderly confusion of detached spores (FIG. 2, *D, a-z*; *E, a-w*). Many of the spores soon germinated by putting forth a germ tube somewhat obliquely from the basal or the distal end (FIG. 2, *F*).

Although in some measure sexual reproduction took place rather early through union of gametangia borne on branches contributed from two mycelial hyphae (FIG. 1, *L, a, b*), it proceeded far more briskly after the surface of the substratum was liberally strewn with detached conidia. In the species here concerned, as in many related forms, most units of sexual apparatus originated from pairs of gametangia whereof one came from a germinating conidium (FIG. 1, *M, a*; *N, a*; *O, a*. FIG. 2, *Ga, a*; *H, a*; *I, a*) while the other came from a mycelial filament (FIG. 1, *M, b*; *N, b, c*; *O, b*. FIG. 2, *G, b*; *H, b*; *I, b*). The paired gametangia appeared to become delimited by basal cross-walls (FIG. 1, *L, s*; *M, s*; *N, s*; *O, s*. FIG. 2, *G, s*; *H, s*; *I, s*) at about the same time they began to fuse at their tips. Sometimes a gametangium contributed from the mycelium was found borne on a branch so short as to appear virtually sessile on the mycelial hypha (FIG. 1, *N, c*. FIG. 2, *G, b*; *H, b*); frequently it was borne on a branch 5 to $25\ \mu$ in length

FIG. 2. *Acaulopage baculispora*.

(FIG. 1, *L*, *a*, *b*; *M*, *b*; *O*, *b*); and occasionally it terminated a branch about 100 μ long (FIG. 1, *N*, *b*). Gametangia of conidial origin often were borne on a short proximal portion of the germ hypha (FIG. 1, *M*, *a*), but frequently, again, they included a portion of the spore itself (FIG. 2, *G*, *a*; *I*, *a*), and sometimes, indeed, the entire spore seemed to function as a sexual cell (FIG. 1, *O*, *a*. FIG. 2, *H*, *a*). Often a conidium collaborated in the production of two units of sexual apparatus. In these instances it would sometimes extend a zygomorphic germ tube from each end (FIG. 2, *I*, *a*), but at other times it would extend one such germ tube from an end position while putting forth the other from a median position (FIG. 1, *N*, *a*).

After the paired gametangia had fused, one of them—very commonly the one of mycelial origin—would form a globose swelling (FIG. 1, *M*, *c*) usually at a distance of 5 to 10 μ from the place of union (FIG. 1, *M*, *u*). This swelling continued to expand (FIG. 1, *L*, *c*. FIG. 2, *H*, *c*; *I*, *c*, *d*) until the remaining portion of the fusion cell was empty of protoplasmic material. The spherical zygosporangium thus brought into being (FIG. 1, *N*, *d*) then underwent the usual internal changes whereby its cellular contents were converted into a zygospore with a thick, boldly verrucose, distinctly yellowish wall. In mature units of sexual apparatus the zygospore, loosely enveloped by the somewhat collapsed sporangial membrane, contained a spherical protoplast wherein a granular parietal layer surrounded a central reserve globule, or sometimes two reserve globules (FIG. 1, *P*, *a-p*. FIG. 2, *J*, *a-l*). Often, besides, a smaller lustrous body, apparently corresponding to the refringent body present in mature oöspores of many oömycetes, seemed indistinctly visible in the granular layer.

In the genus *Acaulopage*, to which the fungus is manifestly referable, it invites comparison especially with the three species I have described earlier under the binomials *A. macrospora* (2: 189-191), *A. stenospora* (9: 256-258), and *A. ischnospora* (12: 263-269), all of which give rise to narrow erect conidia on short sterigmata. It is clearly distinct from *A. ischnospora* by reason of its shorter conidia and their lack of an empty membranous appendage at the tip. From *A. stenospora* it differs very noticeably in the larger diameter of its conidia, for these bodies measure, on

an average, about $1.9\ \mu$ in greatest width, whereas the average for the corresponding dimension in *A. stenospora* is about $1.4\ \mu$. From the conidia of *A. macrospora* those of the present fungus differ in their lack of any evident tendency either toward distal bifurcation or toward evacuation of contents from one of the ends. Besides, they generally taper upward less markedly, so that despite some measure of intergradation with respect to dimensions, they present a rather different appearance because of their more typically filamentous or rod-like shape. Their outward resemblance to walking sticks suggests for the fungus a specific epithet compounded in part of a word meaning "staff."

Acaulopage baculispora sp. nov.

Mycelium effusum; hyphis continuis, incoloratis, filiformibus, parce ramosis, plerumque $1-2\ \mu$ crassis, ad animalia minuta inhaerentibus, pelliculam eorum perforantibus, haustorium (interdum 2 haustoria) intus evolvitibus quod protoplasma exhaurit; haustorio pedicellato, pedicello vulgo $2-5\ \mu$ longo, $0.6-1\ \mu$ crasso, apice abrupte latescente, bis usque quater repetite bifurco, ita $4-16$ (interdum plures) ramulos assumentes divaricatos $2-12\ \mu$ longos, $1-1.4\ \mu$ crassos ferente. Sterigmata inter se saepe $5-50\ \mu$ distantia, erecta, plerumque $1-3.5\ \mu$ alta, basi $1-1.5\ \mu$ crassa, sursum attenuata, apice circa $0.6\ \mu$ vel $0.7\ \mu$ crassa, unicum conidium ferentia; conidiis erectis, incoloratis, aliquid filiformibus, $21-62\ \mu$ longis, $1.8-2.1\ \mu$ latis, utroque parvulum attenuatis, apice abrupte rotundatis. Hyphae zygosporiferae irregulariter flexuosae, ambae interdum ex duabus hyphis mycelii exeuntes sed saepissime altera ex hypha mycelii altera ex conidio germinanti oriunda, quisque gametangium vulgo $10-20\ \mu$ longum, $1.2-2.5\ \mu$ crassum ferens. Zygosporangia primo levia, sphaeroidea, plerumque $7-12\ \mu$ crassa, membrana eorum in maturitate circa zygosporam laxè collapsa; zygospora aliquantum flavida, globosa, $6-11\ \mu$ crassa, valde verrucosa, membrana ejus $1-2.2\ \mu$ crassa, cellulam viventem $3.5-6.5\ \mu$ crassam circumdante.

Amoebas $20-40\ \mu$ latas capiens consumensque habitat in herbis coacervatis putrescentibus prope Mayo, Maryland.

Mycelium spreading; vegetative hyphae aseptate, colorless, filamentous, sparingly branched, mostly $1-2\ \mu$ wide, adhering to minute animals, penetrating the pellicle of each animal thus captured, and intruding a haustorium (sometimes 2 haustoria) to appropriate the protoplasmic contents; haustoria pedicellate, the pedicel commonly $2-5\ \mu$ long and $0.6-1\ \mu$ wide, abruptly enlarging and bifurcating 2 to 4 times and thus bearing 4 to 16 (sometimes more) divergent assimilative branches $2-12\ \mu$ long and $1-1.4\ \mu$ wide. Sterigmata arising from procumbent hyphae often at intervals of $5-50\ \mu$, usually $1-3.5\ \mu$ high, $1-1.5\ \mu$ wide at the base, tapering upward to a

width of $0.6\text{--}0.7\ \mu$ at the tip, whereon is borne erectly a single conidium. Conidia colorless, somewhat filamentous, $21\text{--}62\ \mu$ long, $1.8\text{--}2.1\ \mu$ wide, tapering only slightly toward both ends, abruptly rounded at the tip, when detached somewhat rounded at the base. Zygomorphic hyphae often irregularly flexuous, both of a pair occasionally arising from separate mycelial filaments, but much more often only one of them arising from a mycelial filament, the other being supplied from a germinating conidium, each furnishing a gametangium commonly $10\text{--}20\ \mu$ long and $1.2\text{--}2.5\ \mu$ wide; zygosporangium formed usually $5\text{--}10\ \mu$ from place of union between gametangia, at first smoothly subspherical and mostly measuring $7\text{--}12\ \mu$ in diameter, but its envelope at maturity collapsing loosely about the zygospore; the latter somewhat yellowish, globose, $6\text{--}11\ \mu$ in diameter, boldly verrucose, having a wall $1\text{--}2.2\ \mu$ thick which surrounds a living cell $3.5\text{--}6.5\ \mu$ in diameter.

Capturing and consuming a species of *Amoeba* commonly $20\text{--}40\ \mu$ wide it occurs in heaped decaying herbaceous plants near Mayo, Maryland.

In examining young sexual reproductive apparatus of *Acaulopage baculispora* (FIG. 2, I, e) as well as of other small members of the Zoöpagaceae I have been unable to see in some conjugating elements any cross-wall so placed that it might be considered to delimit a gametangium. While failure to observe a delimiting septum may often be held attributable to optical difficulties frequent in the study of minute objects, the possibility is not to be dismissed that in some instances a special delimiting septum may be absent. The function of such septa in the Zoöpagaceae seems a little problematical since in most members they evidently do not isolate the protoplasm of the gametangium from that of the adjoining portion of mycelial branch or adjoining portion of conidium. In most members of the family, as has been intimated previously (11: 26), a pair of conjugating gametangia together contain only a rather small portion of the protoplasmic material eventually needed for the development of the zygosporangium and zygospore; so that of necessity the larger portion must enter the fusion cell after cross-walls have been formed. It may be presumed, therefore, that in the beginning, at least, the delimiting walls here very probably are not complete partitions, but like the centrally perforated cross-walls in the higher fungi (1: 75-167), have an aperture large enough

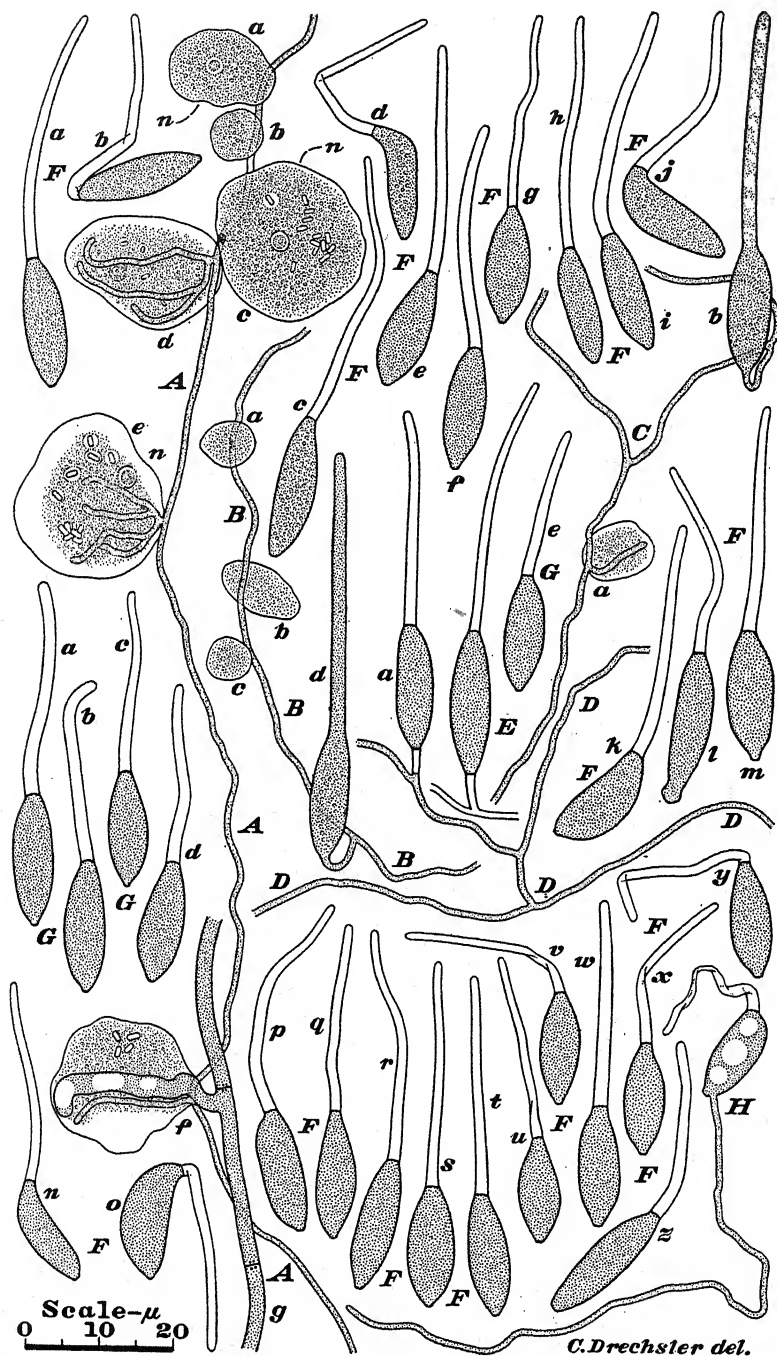
for slow movement of protoplasm—a feature which assuredly need not be shared by the cross-walls laid down in the progressive evacuation of mycelial hyphae and conidia (FIG. 2, *A*). If the apertures inferred from the sequence of developmental events should be more or less variable with respect to size, it appears possible, in view of the narrowness of hyphae in most Zoöpagaceae, that now and then a wall might for a time consist of a peripheral annular thickening so minute as to escape detection. Indeed, it is conceivable that with very narrow hyphae the measure of seclusion ordinarily provided by delimiting septa might perhaps be available here and there without any special partitions being required.

A SPECIES OF ACAULOPAGE PRODUCING ELLIPSOIDAL CONIDIA WITH
LONG APICAL APPENDAGES

A maize-meal-agar plate culture which after being overgrown by *Pythium helicoides* Drechsl. had been further planted with decaying leaves collected on August 20, 1946, from the floor of deciduous woods near Mayo, Maryland, showed in eighteen days a species of *Acaulopage* reminiscent of the two forms I described earlier under the binomials *A. ceratospora* (2: 193–195) and *A. cercospora* (5: 371–374). Its scanty mycelium consisted of colorless, aseptate, sparingly branched hyphae measuring for the most part slightly less than $1\ \mu$ in width (FIG. 3, *A–D*). To these hyphae were found attached at irregular intervals small specimens of *Amoeba* (FIG. 3, *A*, *a–f*; *B*, *a–c*; *C*, *a*) varying in diameter from 6 to $21\ \mu$ when drawn into a somewhat rounded form. As the captured animals, like many other of the more minute *Amoebae* taken by predaceous hyphomycetes, seemed rather lacking in distinctiveness, they could not be satisfactorily identified. They were each enveloped by a very delicate pellicle, and they bore frequently a variable number of bacteria, which sometimes were visibly surrounded by small digestive vacuoles (FIG. 3, *A*, *d–f*). Within the larger specimens a globose or slightly elongated body measuring commonly 2.5 to $3\ \mu$ in total length and 2 to $2.5\ \mu$ in width (FIG. 3, *A*: *a*, *n*; *c*, *n*; *e*, *n*) seemed interpretable as a nucleus with a relatively large endosome. Many of the better developed captives (FIG. 3, *A*, *d*, *e*) were found invaded by a basally ramified bush-like haustorium composed of three or four assimilative branches

having about the same width as the mycelial hyphae. The smaller captives were often found invaded by only a single assimilative branch (FIG. 3, C, a). The delicate phycomycete was likewise restricted to intrusion of a single assimilative branch when, as occasionally happened, one of the larger specimens (FIG. 3, A, f), soon after being captured, was invaded adventitiously by a robust branch extended from a neighboring mycelial filament (FIG. 3, A, g) of the nematode-capturing hyphomycete *Dactylaria thaumasia* Drechsl. (6: 518-523) also present in the culture. Loss of protoplasm continued in all invaded animals until at a final stage of depletion the contents of the haustorium were withdrawn backward into the parent hypha; whereupon the empty tubular membranes soon faded from sight, together with the empty pellicle surrounding them.

Thus amply nourished on living animals the delicate phycomycete gave rise here and there to comparatively robust conidia. In initiating asexual reproduction the mycelial hyphae would put forth short branches that soon widened out rather abruptly to form an erect elongated-ellipsoidal part often about $15\ \mu$ long and from 5 to $6\ \mu$ wide. Through continued but markedly narrowed growth at its apex this swollen part came to be surmounted by a distal beak commonly 20 to $37\ \mu$ long and 1.3 to $1.9\ \mu$ wide (FIG. 3, B, d; C, b). At first the erect structure was filled uniformly with protoplasm throughout its length (FIG. 3, B, d). Later the beak began to show a vacuolated condition (FIG. 3, C, b); and this condition became more and more pronounced until all the protoplasmic material had been withdrawn from the beak into the swollen part below. A retaining wall was then formed at the base of the empty tubular membrane. At about the same time, if not earlier, another cross-wall was laid down between the swollen part and its supporting stalk (FIG. 3, D, a; E) which meanwhile, like the apical beak, had been emptied of contents. At this cross-wall separation took place on slight disturbance. Eventually numerous conidia consisting individually of an elongated-ellipsoidal living cell together with an empty tubular apical appendage were found strewn about in some abundance (FIG. 3, F, a-z; G, a-e). These detached conidia germinated rather freely by extending, often from a position close to the basal hilum (FIG. 3, H), a delicate vegetative

FIG. 3. *Acaulopage gyrinodes*.

hypha evidently capable of capturing any suitable *Amoeba* that might come into contact with it.

Among the species of *Acaulopage* hitherto described only *A. ceratospora* and *A. cercospora* are distinguished by conidia resembling those of the present fungus in having a swollen living cell surmounted by a single narrower tubular appendage. In the present fungus the living cell is scarcely two-thirds as long as in *A. ceratospora*. The apical appendage, too, is shorter than in *A. ceratospora* in approximately the same proportion, besides tapering less pronouncedly; while the basal appendage commonly present in *A. ceratospora* is absent here. By way of contrast the conidia of the present fungus exceed those of *A. cercospora* in all dimensions; the living cell in the former being longer and wider than in the latter by more than a half, while the apical appendage in the former appears about twice as long and three times as wide as in the latter. The fungus obviously represents a new species. A specific term meaning "like a tadpole" may prove helpful in recalling the distinctive shape of its conidia.

***Acaulopage gyrinodes* sp. nov.**

Mycelium sparsum; hyphis continuis, incoloratis, filiformibus, parce ramosis, plerumque $0.8-1\ \mu$ crassis, ad animalia minuta inhaerentibus, pelliculam eorum perforantibus, haustorium intus evolventibus quod protoplasma exhaurit; haustorio interdum in ramo simplici constante, interdum arbusculiformi tum in 2-4 ramis consistente; ramis assumptibus $5-25\ \mu$ longis, $0.8-1\ \mu$ latis. Conidia incolorata, erecta, ex ramulis saepe $2-10\ \mu$ longis oriunda, in duabus partibus constantia; pars supera vacua, plerumque $20-37\ \mu$ longa, basi $1.3-1.9\ \mu$ lata, sursum parvulum attenuata, apice $1-1.6\ \mu$ lata et ibi abrupte rotundata, saepe plus minusve marcida vel collapsa; pars infera protoplasmatis repleta, plerumque elongato-ellipsoidea, vulgo recta sed interdum curvata, $14-20\ \mu$ longa, $4-6.5\ \mu$ lata.

Amoebas saepe $6-21\ \mu$ latas capiens consumensque habitat in foliis arborum (ex magna parte *Quercus*, *Corni*, *Liriodendri*, *Aceris*) putrescentibus prope Mayo, Maryland.

Mycelium sparse; vegetative hyphae aseptate, colorless, filamentous, sparingly branched, $0.8-1\ \mu$ wide, adhering to minute animals, penetrating the pellicle of each animal thus captured, and intruding a haustorium to appropriate the protoplasmic contents; haustorium sometimes consisting of a single assimilative branch, but more often consisting of 2 to 4 assimilative branches, $5-25\ \mu$ long and $0.8-1\ \mu$ wide, in bush-like arrangement. Conidia color-

less, erect, arising singly from branches $2-10\ \mu$ long, each spore consisting of two parts: an upper empty tubular part, $20-37\ \mu$ long, $1.3-1.9\ \mu$ wide below, $1-1.6\ \mu$ wide above, bluntly rounded at its tip, often becoming more or less collapsed; and a lower living part usually of elongated-ellipsoidal shape, usually straight but occasionally somewhat curved, $14-20\ \mu$ long, and $4-6.5\ \mu$ wide.

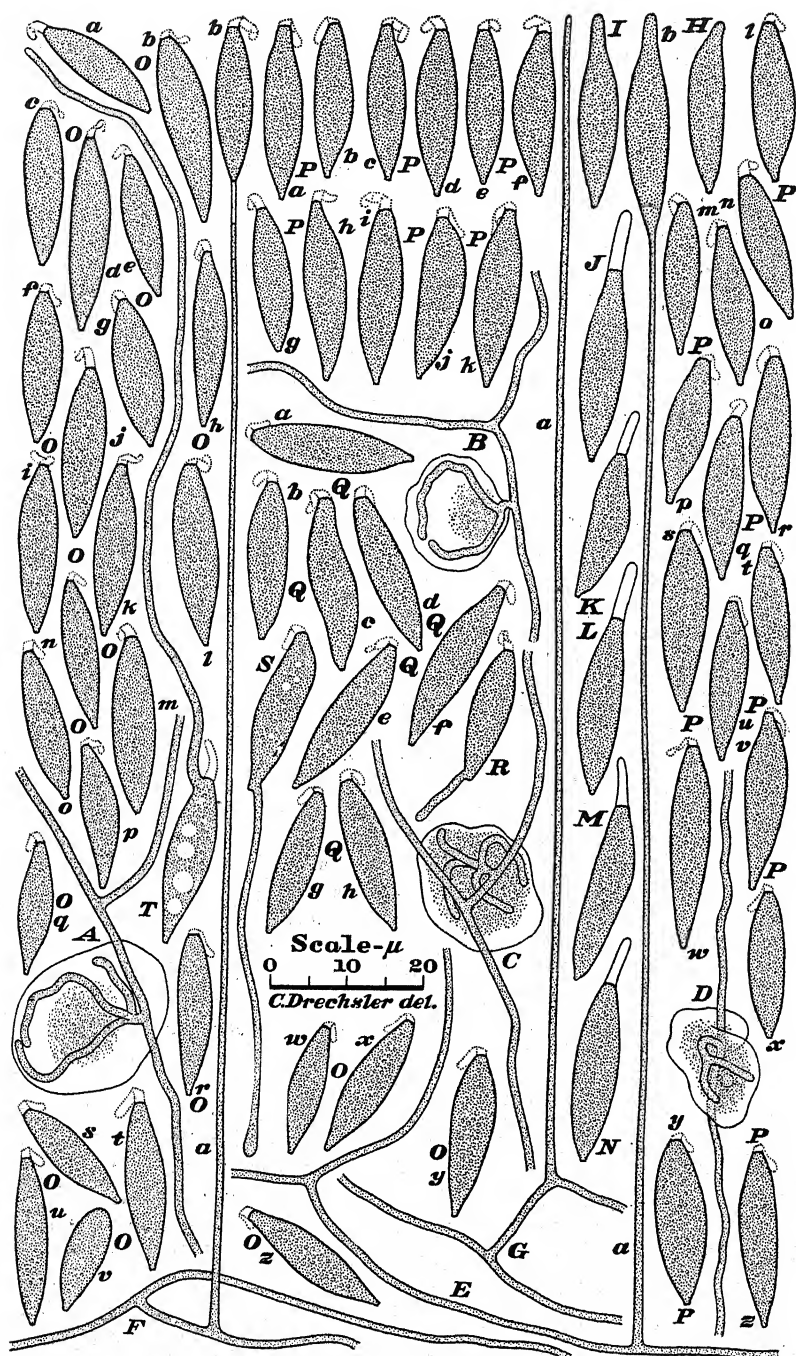
Capturing *Amoebae* often $6-21\ \mu$ wide it occurs in decaying leaves of deciduous trees (including species of *Quercus*, *Cornus*, *Liriodendron*, and *Acer*) near Mayo, Maryland.

A STYLOPAGE PRODUCING DISTALLY APPENDAGED CONIDIA ON TALL
SLENDER CONIDIOPHORES

Among the members of the genus *Stylopage* that have been set forth as subsisting by capture of *Amoebae*, the two species I described under the binomials *S. araea* (2: 199-201) and *S. rhynchospora* (8: 394-397) are distinctive especially by reason of the unusual height—commonly about $200\ \mu$ —of the slender conidiophores on which they produce singly their much wider ellipsoidal conidia. In the stature of their conidiophores as well as in the shape of their conidia these species have supplied a transition to the nematode-capturing forms *S. hadra* Drechsl. (3) and *S. leiohypha* Drechsl. (4) whose robust dimensions might otherwise have made them seem rather alien to the predaceous Zoöpagaceae. A third species manifestly belonging in the same category with *S. araea* and *S. rhynchospora* was recently obtained from leaf mold kindly collected by A. W. Rakosy in oak woods near Frederick, Maryland, on January 15, 1947. The new fungus came to light in several maize-meal-agar plate cultures which after being overgrown by *Pythium irregulare* Buisman had been further planted with small quantities of the forest detritus. When first observed twenty-two days after the leaf mold was added, it was already sporulating somewhat extensively on the agar substratum and must therefore have grown out from the partly decayed oak-leaf fragments nearby, several days—probably between five and ten days—earlier.

The scanty mycelium of the new fungus consisted of meagerly branched aseptate hyphae, mostly about $1\ \mu$ wide, along which

specimens of *Amoeba* commonly measuring 10 to 20 μ across were found attached at variable intervals (FIG. 4, A-D). Most of the captured animals were wholly or in large part depleted of their protoplasm; this depletion having evidently been accomplished by rangy bush-like haustoria consisting individually of two to five assimilative branches of about the same width as the parent hyphae. Mycelial filaments on the surface of the agar substratum gave rise in scattered positions to erect solitary conidiophores (FIG. 4, E, a) which from a basal diameter slightly in excess of 1 μ tapered upward very gradually until at a height of approximately 150 μ , where their width often measured only 0.6 or 0.7 μ , they expanded into a rather massive elongated-ellipsoidal or somewhat fusiform part with a narrowing apical beak (FIG. 4, E, b). Later a cross-wall was formed at the base of the swollen part, and the beak was evacuated of contents; so that the individual conidiophore (FIG. 4, F, a), now delimited distally, came to support aloft a fusiform or elongated-ellipsoidal conidium (FIG. 4, F, b) with a membranous apical appendage. Frequently here, as also in *Stylopage araea*, a short distal portion of the conidiophore was seen to be empty of protoplasmic contents while the conidium was still attached (FIG. 4, F, a). In some instances where disarticulation had probably been hastened by the disturbance entailed in mounting material under a coverglass, denuded conidiophores were found filled with protoplasm throughout their length (FIG. 4, G); and prematurely detached conidia likewise were seen filled to the tip of the beak (FIG. 4, H, I). Where the conidia had matured normally, however, the beak seemed regularly represented by an empty tubular appendage often from one-fourth to one-third as long as the living cell. The appendages were clearly visible, though usually in a badly collapsed condition, when undisturbed cultures were examined microscopically by means of a dry objective. In moist preparations they occasionally were revealed with distinctness and in their original shape (FIG. 4, J-N), but more often in such mounts their diaphanous character and badly collapsed condition made them difficult to see even though the truncate distal end of the living cell left no doubt as to their presence (FIG. 4, O, a-z; P, a-z; Q, a-h). Germination of detached conidia was found to take place by emission of a germ hypha close to the basal hilum (FIG. 4,

FIG. 4. *Stylopaga rhinacra*.

R, S) or, in other instances, close to the wall setting off the empty appendage (FIG. 4, T).

Development of secondary conidia on germ conidiophores put forth erectly from fallen conidia—a type of repetitional reproduction frequent in *Stylopage rhynchospora*—has never been observed in the new fungus. Nor have conidia of the new fungus ever been observed bearing at the tip any adhesive material such as is present in the yellow globular masses whereby conidia of *S. rhynchospora* often cohere apically in pairs. Owing mainly to their lesser width they are of noticeably more slender shape than the conidia of *S. rhynchospora*. In comparison with the consistently unappendaged conidia of *S. araea* they show little difference with respect to width, but because of their generally greater length offer, again, conspicuously more slender proportions.

A term compounded of two words meaning “withered” and “tip,” respectively, may serve conveniently as an epithet for the fungus in recalling its collapsed conidial appendage.

Stylopage rhicnacra sp. nov.

Mycelium sparsum; hyphis continuis, incoloratis, filiformibus, parce ramosis, saepe $0.9\text{--}1.3\ \mu$ crassis, ad animalia minuta inhaerentibus, pelliculam eorum perforantibus, haustorium intus evolventibus quod protoplasma exhaurit; haustorio arbusculiformi, in aliquot (saepe 2–5) ramis assumptibus vulgo $10\text{--}20\ \mu$ longis, circa $1\ \mu$ crassis constante. Hyphae fertiles incoloratae, simplices, erectae, saepe $140\text{--}175\ \mu$ longae, basi $1\text{--}1.3\ \mu$ crassae, sursum leviter attenuatae, apice $0.6\text{--}0.7\ \mu$ crassae, ibi unicum conidium ferentes; conidiis vulgo in cellula viventi et appendice vacua terminali consistentibus: cellula viventi incolorata, elongato-ellipsoidea vel fusiformi, $17\text{--}27\ \mu$ longa, $4.5\text{--}6.5\ \mu$ lata, basi acutula, sursum aliquid applanata; appendice vacua vulgo $5\text{--}8\ \mu$ longa, basi $1.2\text{--}1.8\ \mu$ lata, nunc cylindracea nunc sursum attenuata, apice rotundata, saepissime valde marcida et collapsa.

Amoebas saepe $10\text{--}20\ \mu$ latas capiens consumensque habitat in foliis *Quercus* putrescentibus prope Frederick, Maryland.

Mycelium scanty; vegetative hyphae aseptate, colorless, sparingly branched, often $0.9\text{--}1.3\ \mu$ wide, adhering to minute animals, penetrating the pellicle of each animal thus captured and intruding a haustorium to appropriate its protoplasmic contents; haustorium bush-like, consisting of several (often 2 to 5) assimilative branches commonly $10\text{--}20\ \mu$ long and about $1\ \mu$ wide. Conidiophores colorless, simple, erect, often $140\text{--}175\ \mu$ long, $1\text{--}1.3\ \mu$ wide at the base, tapering very gradually upward, $0.6\text{--}0.7\ \mu$ wide at the tip whereon

is borne a single conidium. Conidia consisting of a living cell and an empty terminal appendage—the living cell colorless, elongate-ellipsoidal or somewhat spindle-shaped, 17–27 μ long and 4.5–6.5 μ wide, somewhat acute at the narrow basal end, roundly truncate at the wider distal end—the empty terminal appendage commonly 5–8 μ long and 1.2–1.8 μ wide at the base, sometimes virtually cylindrical but at other times tapering upward perceptibly, always rounded at the tip, very often withered and collapsed.

Capturing and consuming *Amoebae* 10 to 20 μ wide it occurs in decaying leaves of *Quercus* near Frederick, Maryland.

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EXPLANATION OF FIGURES

FIG. 1. *Acaulopaga baculispora*, drawn to a uniform magnification with the aid of a camera lucida; $\times 1,000$ throughout. A, B, Portions of mycelial hyphae from each of which a young haustorium has been intruded into a captured specimen of the *Amoeba* sp. most commonly taken as prey. C–E,

Branched portions of mycelium from each of which a fairly well developed haustorium has been intruded into a captured specimen of the *Amoeba* sp. most frequently serving as prey. *F, G*, Branched portions of mycelium from each of which two haustoria have been intruded into a separate specimen of the *Amoeba* sp. most frequently taken as prey; in each instance one haustorium, *a*, is in young condition whereas the other, *b*, is in further developed condition. *H, I*, Portions of mycelial hyphae, each with a richly branched haustorium in a captured *Amoeba* that has been very largely expropriated of its protoplasm. *J, K*, Portions of hyphae possibly belonging to the same predaceous fungus; from each portion a young haustorium has been intruded into a specimen of an *Amoeba* sp. that was not frequently observed taken as prey. *L*, Young unit of sexual reproductive apparatus produced by two mycelial hyphae, *a* and *b*; these hyphae have given off branches bearing gametangia whose conjugation is being followed by development of a zygosporangium, *c*. *M*, Unit of sexual reproductive apparatus contributed jointly by a germinating conidium, *a*, and a mycelial hypha, *b*; the paired gametangia have fused, and a zygosporangium, *c*, is beginning to develop in the gametangium supplied from the mycelial hypha. *N*, Two units of sexual reproductive apparatus formed through interaction of a germinating conidium, *a*, with two mycelial hyphae, *b* and *c*; the conidium has given rise to two gametangia on separate germ hyphae; these gametangia have conjugated with two others borne separately on branches given off by the mycelial hyphae; in the latter gametangia the zygosporangia *d* and *e* are being formed. *O*, Mature unit of sexual reproductive apparatus contributed jointly from a conidium, *a*, and a mycelial hypha, *b*; the zygosporangium *c*, which contains a mature zygosporangium, was formed in the gametangium supplied from the mycelial hypha. *P*, Mature zygosporangia, *a-p*, each loosely surrounded by the slightly collapsed zygosporangial envelope; the attachment of the envelope to the empty tubular membranes of the gametangia is shown in most instances. (*n*, nucleus of captured *Amoeba*; *s*, cross-wall delimiting gametangium proximally; *u*, place of union between paired gametangia; *v*, contractile vacuole of captured *Amoeba*.)

FIG. 2. *Acaulopage baculispora*, drawn to a uniform magnification with the aid of a camera lucida; $\times 1,000$ throughout. *A*, Portion of branched prostrate hypha active in asexual reproduction: *a*, erect conidium in early stage of growth; *b*, erect conidium probably full grown or nearly full grown, though not yet delimited at its base; *c-g*, five sterigmata, each bearing erectly a basally delimited conidium. *B*, Portion of branched prostrate mycelial hypha bearing five sterigmata, *a-e*, each bearing an erect conidium. *C*, Portion of prostrate mycelial hypha bearing five denuded sterigmata, *a-e*. *D* (*a-z*), *E* (*a-w*), Detached conidia, showing usual variations in size and shape. *F*, Detached conidium germinating obliquely from its distal end. *G*, Young unit of sexual reproductive apparatus contributed from a germinating conidium, *a*, and a mycelial hypha, *b*. *H*, Somewhat older unit of sexual reproductive apparatus likewise contributed from a germinating conidium, *a*, and a mycelial hypha, *b*; *c*, young growing zygosporangium. *I*, Two units of sexual reproductive apparatus resulting from conjugation of two gametangia supplied from the germinating conidium *a* with two other gametangia supplied from the mycelial hypha *b*; in the latter two gametangia the zygo-

sporangia *c* and *d*, respectively, are being formed; one of these gametangia, *c*, is not yet visibly delimited by a cross-wall. *J*, Mature zygospores, *a-l*, each loosely surrounded by the zygosporangial envelope (*s*, cross-wall delimiting gametangium proximally; *u*, place of union between paired gametangia).

FIG. 3. *Acaulopage gyrinodes*, drawn to a uniform magnification with the aid of a camera lucida; $\times 1,000$ throughout. *A*, Portion of mycelial hypha with six captured *Amoebae*, of which three, *a-c*, have not yet been invaded, whereas the others, *d-f*, have been partly depleted of their protoplasm by haustorial branches intruded into them in numbers of three, four, and one, respectively; one captive, *f*, has been further invaded by a stout branch from a mycelial hypha, *g*, of *Dactylaria thaumasia*; *n*, nucleus of captured animal. *B*, Prostrate mycelial hypha on which three small *Amoebae*, *a-c*, are held captive; the hypha has given rise on a short lateral branch to an erect conidium, *d*, which though full grown has not yet been delimited at its basal end, and is still uniformly filled with protoplasm throughout its apical prolongation. *C*, Portion of prostrate mycelial hypha which has extended an assimilative branch into a small captured *Amoeba*, *a*, and has produced an erect conidium, *b*, from whose vacuolated apical prolongation the protoplasmic contents are being withdrawn. *D*, Portion of prostrate mycelium bearing a mature erect conidium, *a*, whose ellipsoidal living cell is delimited basally from the empty stalk and distally from the empty elongate apical appendage. *E*, Empty portion of prostrate hypha bearing a mature appendaged conidium on a short empty stalk. *F* (*a-s*), *G* (*a-e*), Mature detached conidia showing variations in size and shape of the living cell as well as of the empty apical appendage. *H*, Detached conidium that has put forth a germ hypha obliquely from a position near its base.

FIG. 4. *Stylopaga rhinacra*, drawn to a uniform magnification with the aid of a camera lucida; $\times 1,000$ throughout. *A-D*, Portions of mycelial hyphae from each of which a branched haustorium has been extended into a captured *Amoeba*; all the captives have been largely expropriated of their protoplasmic contents. *E*, Portion of prostrate hypha from which has been sent up a slender erect conidiophore, *a*, that is still continuous distally with the conidium, *b*, whose apical prolongation is still filled uniformly with protoplasm. *F*, Portion of prostrate mycelium from which has been sent up an erect conidiophore, *a*, bearing a mature conidium, *b*, that is provided with an empty apical appendage. *G*, Portion of prostrate hypha with an erect conidiophore, *a*, not supporting a conidium. *H, I*, Detached conidia in which the apical beak is still filled with protoplasm. *J-N*, Detached conidia wherein the empty apical appendage has not collapsed or shriveled. *O* (*a-s*), *P* (*a-s*), *Q* (*a-h*), Random assortment of detached conidia showing usual variations in size and shape of the living cell; the apical appendage here being in varying degree collapsed or shriveled, and often barely discernible. *R, S*, Detached conidia, each with a germ tube arising in close proximity to its base. *T*, Detached conidium with a germ hypha arising in close proximity to its distal end.

A COMMON ANTIGENIC FACTOR IN DIFFERENT SPECIES OF SPOROTRICHUM

H. I. LURIE¹

Dodge (8) describes six human and one equine species of *Sporotrichum*. The human ones are *S. Grigsbyi*, *S. Fonsecai*, *S. asteroides*, *S. cracoviense*, *S. Carougeau*, *S. Jeanselmei* and *S. Schenckii* and the equine species is *S. equi*. He recognizes four varieties of *S. Schenckii*, namely vars. *Beurmanni*, *Greconis*, *Councilmani* and *Fiocci*. His key to the species is based on liquefaction of gelatine, coagulation of milk, and pigment formation. However, he states that "the delimitation of species in this genus is very difficult. Color, which has often been used not only to separate species but even genera, has been shown by Davis (6) to be variable. Practically all strains that he tried formed pigment when first isolated. . . . Albino strains frequently occur and remain fixed even after passage through an experimental animal. Meyer and Aird (10), after a study of eighteen strains, found some differences in carbohydrate fermentation, but these were not sufficiently constant or correlated with other characters to warrant separation into species." Davis (5) found that the production of chlamydospores was of no value in the differentiation of species.

Meyer (9) states that extensive bacteriologic and serological experiments have proved the identity of the causative organisms in human and animal sporotrichosis, and that the pathogenicity of the equine strain for human beings was observed in an accidental laboratory infection. Davis (4) compared three human and two equine strains by their agglutination reactions and found no differences. Two of his human strains were isolated in America and

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classified as *S. Schenckii*, the third was received from Gougerot in Paris and classified as *S. Beurmanni*. The equine strains were supplied by Dr. Meyer of Philadelphia. In 1921 Davis (7), after a careful study of the original descriptions of *S. Schenckii* and *S. Beurmanni* and comparison of the cultures, concluded that they were identical and that the name *S. Schenckii* should have priority. He also states that *S. Jeanselmei* is probably identical with *S. Beurmanni* but concedes that *S. Dori* and *S. Councilmani* are different. The former has been lost and the latter was unfortunately not available for this study.

The object of this investigation is to establish the antigenic identity of different strains of *Sporotrichum* after the method of Davis (4). The method he employed however was rather laborious. It required eight months to obtain a satisfactory titer in his rabbits. Recently Campbell (3) described a technique for culturing *Sporotrichum* in a "yeast" phase by growing the organism on cystine-agar at 37° C. This appeared to be an ideal method to obtain a concentrated suspension of spores free from mycelium for use as an antigen. Preliminary experiments proved this in fact to be the case. The method finally adopted is described below.

Sixteen strains of *Sporotrichum* were selected. Their origins and classifications are as follows:

- No. 1. *S. Schenckii*, human case, Duke Hospital, N. Carolina.
- No. 2. *S. Schenckii*, human case, Duke Hospital, N. Carolina.
- No. 3. *S. Schenckii*, human case, Temple, Texas.
- No. 4. *S. Schenckii*, human case, isolated from spleen, Cincinnati, Ohio.
- No. 5. *S. Schenckii*, human case, New York.
- No. 6. *S. Schenckii*, human case, Virginia.
- No. 7. *S. Schenckii*, human case, Indiana.
- No. 8. *S. Schenckii*, human case, Mexico.
- No. 9. *S. asteroides*, Splendore, Westerdijk, Baarn.
- No. 10. *S. Beurmanni*, M. and Ram, Westerdijk, Baarn.
- No. 11. *Rhinocladium Beurmanni*, Langeron, Paris.
- No. 12. *Rhinocladium equinum*, Sabouraud's collection, Langeron, Paris.
- No. 13. *S. Beurmanni*, isolated from mine timber, South Africa.
- No. 14. *S. Beurmanni*, isolated from mine timber, South Africa.
- No. 15. *S. Beurmanni*, human case contracted in a mine, South Africa.
- No. 16. *S. Beurmanni*, human case contracted in a mine, South Africa.

Numbers 1 to 12 were supplied by Dr. Conant and numbers 13 to 16 were supplied by Brown, Weintraub and Simpson (2) of the

Transvaal Chamber of Mines Timber Research Laboratory. The color of the cultures varied from creamy-white to dark brown, almost black.

All cultures were first grown on Sabouraud's dextrose-agar at room temperature for two weeks. They were then transferred to Difco Cystine-agar slopes and incubated at 37° C. for five to seven days, after which they were transferred to fresh Cystine-agar slopes and incubated for a further five to seven days. At the end of this period the majority of cultures appeared to be quite "yeasty." However, a few required further transfers and one (no. 12) required to be passed through a mouse before a "yeasty" growth was obtained. The growths from four slopes were transferred with a platinum loop to approximately 30 cc. of sterile isotonic saline. This was sufficient to give a milky suspension of spores. Where it was necessary to remove pieces of agar or large clumps of spores the suspension was filtered through several layers of gauze. The spore suspensions were then killed by heating 60° C. for two hours. The density of each spore suspension was measured by centrifugation in Hopkin's tubes and each adjusted by the addition of saline to a concentration of 1:200. From this a suspension of 1:1,000 was made. The former was used for the injection of rabbits, the latter for the subsequent agglutination tests. All suspensions were tested for sterility in the usual manner before use.

Four strains were selected for the production of antiserum, namely Nos. 1, 3, 9 and 12, two being classified as *S. Schenckii*, one as *S. asteroides* and one as *Rhinocladium equinum*.

The rabbits were injected intravenously according to the schedule recommended by Benham (1) for Monilias. On the first day 1 cc. was given, 2 cc. on the second day and 5 cc. on the third day. The same doses were repeated a week later, and the rabbits bled one week after the last injection. One rabbit required a further three injections before a satisfactory titer was obtained. The final titers were between 1:640 and 1:1,280.

Agglutination reactions were carried out with all sixteen strains using each of the four antisera. An amount of 0.5 cc. of a 1:1,000 suspension of the antigen was added to 0.5 cc. of antiserum in dilutions ranging from 1:10 to 1:2,560, the mixtures then shaken and

the racks placed in a waterbath at 37° C. overnight. A control of 0.5 cc. antigen suspension with 0.5 cc. saline was used in each case to detect auto-agglutination and the results were read macroscopically. Where the last tube showing agglutination displayed complete clumping the result was designated by a +; where the last tube showed only partial agglutination the result was designated by a \pm . A summary of the results is given in Table 1.

TABLE 1

Strain	Titer of Agglutination with Antiserum			
	1	3	9	12
1	+1280	+ 320	+ 640	+ 640
2	\pm 1280	+ 640	\pm 640	+1280
3	\pm 1280	\pm 1280	\pm 1280	+ 640
4	\pm 640	+ 320	\pm 640	+1280
5	\pm 640	\pm 1280	\pm 640	\pm 640
6	+1280	+1280	+1280	+ 640
7	\pm 640	+ 320	\pm 640	\pm 640
8	\pm 640	+ 320	\pm 640	+ 640
9	\pm 320	\pm 640	+ 640	\pm 1280
10	+ 640	\pm 640	\pm 640	\pm 1280
11	+ 640	\pm 640	+ 640	+ 640
12	+1280	\pm 640	\pm 1280	+1280
13	\pm 640	+ 640	\pm 1280	\pm 640
14	\pm 1280	+ 640	\pm 1280	+1280
15	\pm 1280	\pm 1280	\pm 1280	+1280
16	\pm 640	+ 320	+ 320	\pm 1280

Each antiserum agglutinated all sixteen strains to approximately equal titers. In no case was any one strain agglutinated in lower titer than the other strains by all the antisera. All sixteen strains examined therefore have a common antigenic factor.

In order to confirm these findings absorption experiments were carried out. Antiserum no. 3 was absorbed with strain no. 9 for a short time (1 cc. antiserum with 4 cc. 1:200 antigen for 2 hours at 37° C.). The antiserum-antigen mixture was then centrifuged and the supernatant fluid used for agglutinating all sixteen strains. The results are shown in Table 2. The titer of the antiserum was slightly lowered for all sixteen strains and to an approximately equal degree. Antiserum no. 1 was absorbed with strain no. 9 for a longer period (18 hours) in the same proportions as above. The supernatant fluid was again used for agglutinating all sixteen

TABLE 2

Strain	Original Titer with Antiserum no. 3	Titer after Absorption with Strain no. 9 (2 hours)
1	+ 320	±160
2	+ 640	±320
3	±1280	±320
4	+ 320	+160
5	±1280	+320
6	+1280	±640
7	+ 320	±160
8	+ 320	+160
9	± 640	+160
10	± 640	+160
11	± 640	+320
12	± 640	±160
13	+ 640	±320
14	+ 640	+320
15	±1280	±320
16	+ 320	±160

strains. The results are shown in Table 3. There is an appreciable and equivalent drop in titer in each case. Similarly antiserum no. 12 was absorbed with strain no. 1. The results are shown in Table 4.

TABLE 3

Strain	Original Titer with Antiserum no. 1	Titer after Absorption with Strain no. 9 (18 hours)
1	+1280	+ 80
2	±1280	±160
3	±1280	±160
4	± 640	± 80
5	± 640	+ 80
6	+1280	+160
7	± 640	± 80
8	± 640	± 80
9	± 320	+ 80
10	+ 640	±160
11	+ 640	± 80
12	+1280	+ 80
13	± 640	+160
14	±1280	+ 80
15	±1280	±160
16	± 640	±160

Finally antiserum no. 12 was absorbed with varying proportions of a 1:2 suspension of strain no. 3. The smallest amount of antigen which had completely absorbed its own antibodies was determined. This was found to be equal proportions of antiserum and a 1:2 suspension of antigen. A further sample of no. 12 antiserum was then absorbed with this amount of strain no. 3.

TABLE 4

Strain	Original Titer with Antiserum no. 12	Titer after Absorption with Strain no. 1 (18 hours)
1	+ 640	± 320
2	+1280	± 320
3	+ 640	± 320
4	+1280	+160
5	± 640	+160
6	+ 640	± 320
7	± 640	± 320
8	+ 640	± 160
9	± 1280	± 320
10	± 1280	+320
11	+ 640	± 160
12	+1280	± 320
13	± 640	± 320
14	+1280	± 320
15	+1280	± 640
16	± 1280	± 320

The absorbed serum was then put up against all sixteen strains. The results are shown in Table 5. It will be seen that there has been practically complete absorption of the agglutinins for all sixteen strains.

TABLE 5

Strain	Original Titer with Antiserum no. 12	Titer after Complete Absorption with Strain no. 3
1	+ 640	—
2	+1280	—
3	+ 640	—
4	+1280	—
5	± 640	—
6	+ 640	—
7	± 640	—
8	+ 640	—
9	± 1280	± 20
10	± 1280	—
11	+ 640	—
12	+1280	—
13	± 640	—
14	+1280	—
15	+1280	± 20
16	± 1280	± 20

All sixteen strains (three species and one variety), therefore, contain a common antigenic factor.

SUMMARY

1. A simple technique for the preparation of *Sporotrichum* antigen, the immunization of rabbits and an agglutination test are described.

2. Sixteen different strains of *Sporotrichum* belonging to at least three species, originating in three continents and varying in color from creamy-white to dark brown, are compared by their serological reactions.

3. *S. Schenckii*, *S. Beurmanni*, *S. asteroides* and *Rhinocladium equi* originating in North and South America, Europe and South Africa all have a common antigenic factor.

4. This common antigenic factor is present irrespective of the degree of pigmentation of the fungus.

5. The *Sporotrichum* growing saprophytically on the timber in the mines in South Africa contains the same antigenic factor as the *Sporotrichum* isolated from the lesions of workers in these mines.

Further absorption experiments are being carried out in order to determine whether the antigenic structures of these species of *Sporotrichum* are identical or merely contain one common factor.

ACKNOWLEDGMENTS

I am indebted to the Duke University for their hospitality and for granting me the facilities to carry out this work; to Dr. Norman F. Conant for his invaluable advice and constant interest; to Miss Jean Y. Biser and Mr. William C. Council for the preparation of the media.

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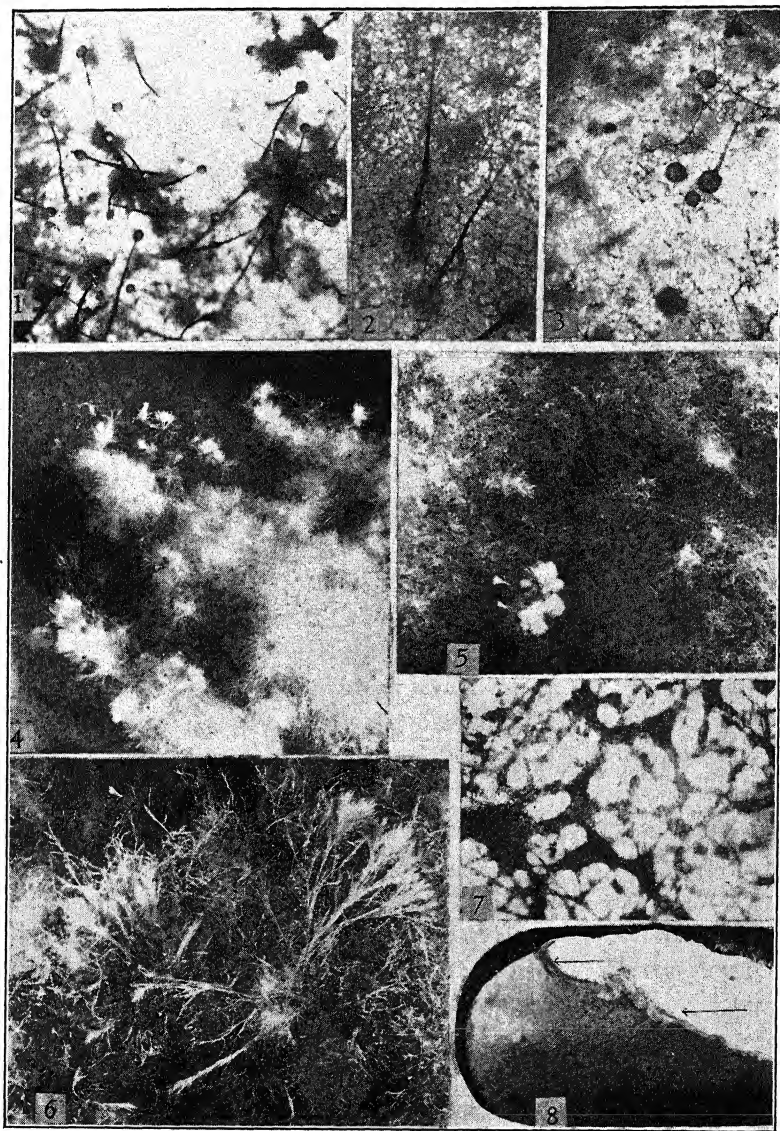
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SPHAERONAEMELLA FIMICOLA (MAR- CHAL) EMEND.: SOME CHARAC- TERISTICS IN CULTURE

DOROTHY PEASE

(WITH 19 FIGURES)

It has been shown for several of the species which were originally assigned to the genus *Sphaeronaema* that the supposed pycnidia are in reality ascocarps (Schwarz, 1922; Elliott, 1923; Sartoris, 1927; Dade, 1928). These species were thereafter removed to the genus *Ceratostomella*, and later to *Ophiostoma* (*Ceratostomella*) (Sydow and Sydow), (Melin and Nannfeldt, 1934). All of the species thus transferred produce black ascocarps. When Nannfeldt and Melin (1934) revised the genus *Ceratostomella* to *Ophiostoma* they suggested the probability that other species of *Sphaeronaema* might eventually be found to belong in it; but these could be only such species as have black fruits. More recently Seeler (1943) found that the fruiting bodies of *Sphaeronaema Helvellae* were in reality ascigerous instead of pycnidial. This species produces light colored, fleshy ascocarps which exclude it from *Ophiostoma* (*Ceratostomella*); furthermore it has hyaline ascospores which exclude it from the dark-spored *Melanospora*. First described by Karsten as *Sphaeria Helvellae*, it was later made by him the type species for a new genus, when it became *Sphaeronaemella Helvellae* (Karsten 1884). Jaczewski (1898) in his monograph on *Sphaeronaema* transferred the *Sphaeronaemellae* to *Sphaeronaema*. However, with the establishment of the ascigerous character of the fruits of *S. Helvellae* it became necessary to remove it from this genus, and Seeler (1943) has returned it to *Sphaeronaemella*, reestablishing the earlier genus with an emended description which places it among the Ascomycetes in the Nectriaceae, near *Melanospora*. Thus a place has been made for other light fruited, hyaline spored species of *Sphaeronaema* which may prove in reality to be Ascomycetes.



FIGS. 1-8. *Sphaeronaemella fimicola*.

The present paper deals with such a light colored *Sphaeronacma* with hyaline spores, whose fruits are ascocarps, not pycnidia. It must therefore join Seeler's *Sphaeronaemella Helvellae* (Karsten). Our fungus is a saprophyte, found on squash, citron, and gourd which has begun to rot on the ground. Though visible growth may be suppressed by dry air or by other molds in the same environment, two asexual forms are sometimes found in the field. At the margin of the rotting area on the fruit delicate tufts of short unbranched hyphae, tipped with curving chains of conidia, erupt through the fruit rind, like a miniature setting of shrubs (FIG. 9). Toward the center of the rotted area the mold forms a weft of snow white mycelium traversed by heavy ropes of conidia which often collect in large moist clumps (FIG. 7). Sexual fruits have not been found in any of our field collections, but develop abundantly in moist chamber preparations and in cultures kept at about 15° C. (FIGS. 1, 2, 3).

CULTURES. This species grows well on agar which contains plant material if the humidity in the culture dish, and the water content of the agar, are both relatively high. Irrigating wells (Pease 1935) have been used to provide these conditions.

MYCELIUM. Mycelial growth is white, the form of the growth varying with the environment. The hyphae are composed of uninucleate cells, 12–16 μ long, and average 2.4 to 3.2 μ in diameter. On sterile squash rind which has been kept thoroughly moist there are produced the delicate tufts of conidiophores which are sometimes seen in the field (FIG. 9). The aerial growth of mycelium with ropes and masses of conidia develops in high humidity and cool temperature, especially from mass conidial and mass ascospore inocula (FIG. 7). Heavy inoculation of agar which contains finely sieved squash rind and slightly lowered water content develops profusely branched white tufts of mycelium often producing no conidia (FIGS. 4, 5, 6). These are especially noticeable in single ascospore strains. Occasionally cultures develop in which the mycelium is sparse, mostly or wholly sterile, and spreading over the entire plate with no distinguishing form.

CONIDIA. These cells are oval, non-septate, with a single nucleus, commonly 12 \times 5–6 μ (FIGS. 16, 17, 18). A longer somewhat curved septate conidium may very rarely be found. All conidia

germinate readily in water, producing from one to as many as six germ tubes. Conidial production varies with the habitat in which the inoculum is placed. Like species of *Ophiostoma* (*Ceratostomella*) described by Münch (1907), Clinton & McCormack (1936), Lagerberg, Lundberg, and Melin (1927), and Goidanich (1935), our species produces cephalosporial, verticillate, "stag-horn" (FIG. 14), and other types of growth. Conidia develop both terminally and laterally. The two most characteristic forms are probably those found in the field, chalaral (FIG. 9) and the long horizontal chains and ropes of conidia described by Münch (1907) for his blue-stain *Ceratostomellae*.

Two types of conidiophores described for various species of *Ophiostoma* and related fungi have not appeared in the hundreds of cultures made from conidial and ascospore inocula during a five year period of study. These are the graphial and penicilloid fructifications. (Cf. Münch 1907, Schwarz 1922, Grossman 1932, and others.)

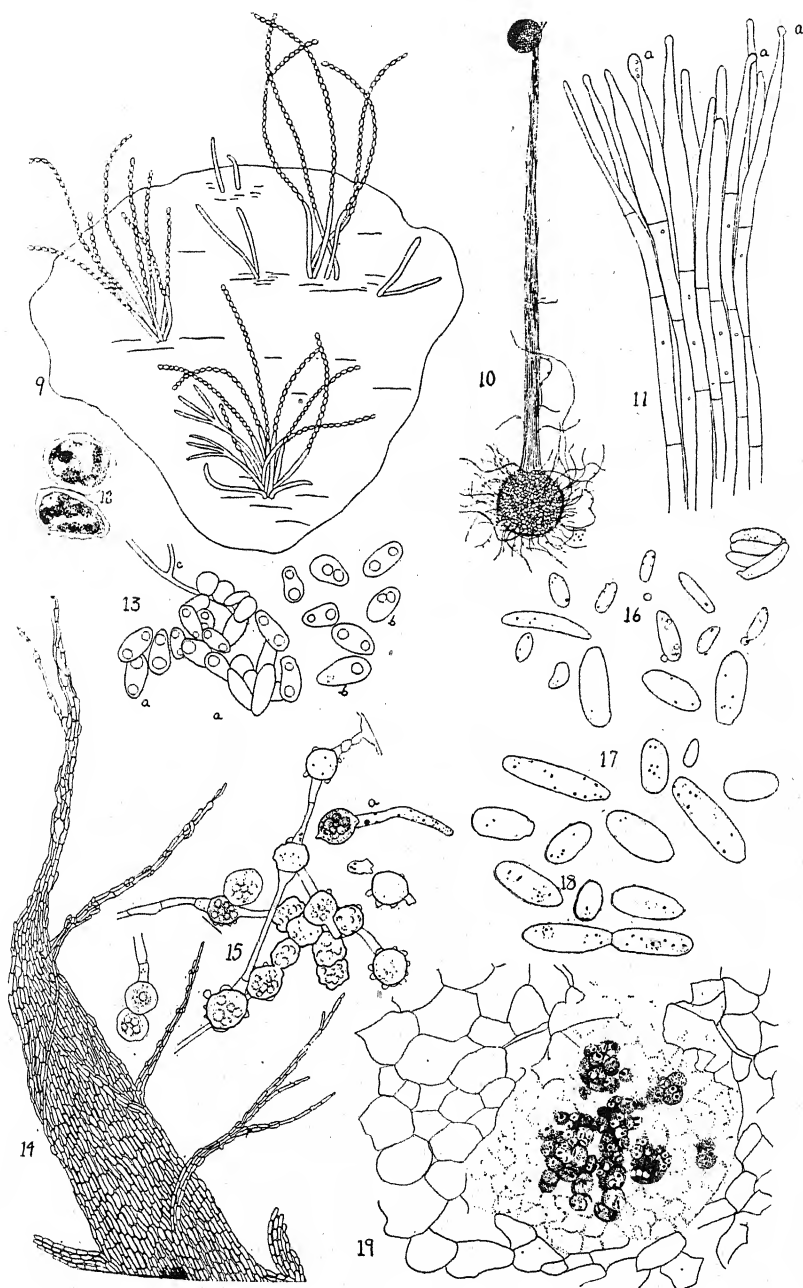
CHLAMYDOSPORES. These structures are conspicuous under the microscope in mycelia produced from mass conidial or mass ascospore inocula. High humidity and moisture and lower temperature favor them. Either terminal or intercalary cells enlarge to a diameter of 8μ (extremes: 5.6 to 16μ), develop a very thick hyaline mucilaginous wall which may remain smooth or bear large warty protuberances (FIG. 15). Their contents are large closely packed globules which are probably neither volutin nor oil, but react more like glycogen. Chlamydospores are especially abundant in the vicinity of the base of the ascocarp; they are often found inside the base of old recumbent beaks. Frequently they are accompanied by a slightly flattened adjacent cell, in appearance resembling a receptacle. They germinate readily in water mounts, producing one or more germ tubes.

YEAST-LIKE CELLS. In old cultures, kept moist and cool, yeast-like cells develop abundantly in the mycelial mat in direct contact with the substrate. These are particularly noticeable in squash rind and maltose agars in the tough layer of pink growth closely adherent to the agar (FIG. 8).

ASCOCARPS. Long-beaked, flask-shaped sexual fruits have developed only in very moist cultures at low temperature (FIGS. 1, 2, 3).

Sterile water dropped by pipette onto the surface of the mycelium is often necessary to stimulate their formation. At their optimum temperature of 15° C. they mature in from six to ten days. They develop more readily in cultures kept in a cold room, with slightly varying day and night temperatures and with alternating daylight and darkness, than they do in cultures kept in a low temperature incubator. This is perhaps due to the increase in the surface-droplets of moisture naturally developing with changing temperature. The ascocarps may be scattered, or clustered densely if water has stood on the substrate or on the mycelium. These structures are usually erect, though they may assume various angles with relation to the substrate, sometimes even lying prone, and buried in the agar (FIG. 3).

The ascocarp is pink, modified to a salmon or rusty shade. "Onion-skin-pink" on plate 28 of Ridgway's (1912) color standards is perhaps the correct shade. The length of the entire ascocarp on fruit rind cultures is 1 to 1.5 or often 2 mm.; occasionally extreme variations occur. The length of the beak is usually five to eight times the diameter of the basal bulb. The beak is tipped with from eight to fifteen spreading setae of varying length, the "crown of eyelashes" of Münch (FIG. 11). The soft bulbous base is composed of pseudoparenchymatous tissue; the inner layers break down to form a gelatinous matrix in which the spores mature (FIG. 19). The mature asci are softly angulated, with a thick gelatinous wall. They are eight spored, evanescent, and disappear soon after the spores are delimited (FIG. 12). The ascospores are oval and usually biguttulate, commonly $4.4 \times 8.8 \mu$ (FIG. 13). Their discharge through the long beak is preceded by the appearance of a clear globule of liquid resting on the setae. If a current of air strikes this droplet before the spores have been discharged into it, the liquid is withdrawn into the interior, to be extruded again later. The spores collect in this terminal drop as they are ejected, making it milky white. At this stage the appearance of a thickly fruited culture is as of many pearls borne on delicate pink pedestals. As the spore clusters dry they become cream or tan colored, but the spores never lose their hyaline character. Often conidia are budded terminally from the setae (FIG. 11), and germinate in situ, the hyphae growing downward to neighboring



FIGS. 9-19. *Sphaeronaemella fimicola*.

ascocarps or to the mycelium, forming delicate festoons, quite characteristic of older culture plates. Within the ascocarp, after the discharge of the spores to the setae, fine mycelial threads grow through the wall of the ascocarp into the gelatinous matrix which may still exist in the basal bulb. These wandering hyphae, together with the absence of the classical club-shaped asci, and the presence of some lingering hyaline oval ascospores in the perithecial cavity, create the distinct illusion of a pycnidial rather than of an ascigerous structure. If these intruding branching hyphae are analogous to paraphyses, they do not resemble them in general appearance.

HETEROTHALLISM. Removal of the slime droplet on the setae to sterile water eventually frees the spores for single spore isolations. Cultures obtained from these single spores are far less vigorous than those from multiple-spore inocula. This species is heterothallic. No ascocarps have ever developed from any of the many single ascospore strains which have been obtained. When such single spore strains, complementary in nature, are crossed it has usually been found necessary to place a large mycelial inoculum from each of the two strains in very close proximity on an agar plate in order to secure perithecia. The physiological explanation has not been determined. Monoascospore strains seem more prone to morphological variation than multiple-spore strains, which fact may have an important bearing on the problem.

HABIT. Four collections of this species have been made from rotting cucurbits from New Jersey and Connecticut, each time after a "wet" summer. Thus far it has not been found in the field after a season of diminished rainfall. It was first noticed in moist chamber preparations of hubbard squash heavily infected with *Mycosphaerella citrullina* (Smith) Grossman; the pink beaks of the ascocarps of our mold rose above a thick expanse of the pink conidia of *Mycosphaerella*. Subsequent to study in pure culture, the characteristic snow white mycelium with its long chains of conidia was later recognized in the field against the dark rind of acorn squash. Mites (*Tarsenema*) are so fond of this fungus that it was some months before a sufficiently exact technique for their exclusion was developed. In cultures to which they at first gained access no ascocarps matured; the initial cells were devoured by the

intruders. The prevalence of such mites on rotting fruits could be one reason for the failure to find ascocarps developing in the field.

PATHOGENICITY. Repeated attempts to grow this fungus on various cucurbit fruits and on fresh twigs have consistently met with failure, though it grows well on the same fruits after they have been autoclaved. Its habit is essentially saprophytic.

SYNONYMS. A search of the literature has yielded one very brief description of a similar mold collected in North America.¹ Ellis and Everhart (1889) described *Sphaeronaemella carnea* as a new species, collected seventeen years earlier from ash bark at Lake Skaneateles, N. Y. We have not been successful in locating any type material of this species. As a check, autoclaved moist twigs (elm) were inoculated with our cultures. These twigs supported good growth of pink perithecia.

Jaczewski (1898) in his monograph on *Sphaeronaema* describes two species which resemble ours: *Sphaeronaema fimicola* (*Sphaeronaemella fimicola* Marchal) and *Sphaeronaema Helvellae* (*Sphaeria Helvellae* Karsten; *Sphaeronaemella Helvellae* Marchal). Through the courtesy of the late Dr. Linder it was possible to make a direct comparison with some of Karsten's original *Sphaeria Helvellae*, Fungi Fennic. no. 674, in the Farlow Herbarium. Our fungus is definitely not the same species. No type material of *Sphaeronaemella fimicola* or of *Sphaeronaema fimicola* could be located either here or abroad (two or three years before the last war), but the Farlow Herbarium has one of von Höhnelt's slides made from material collected by Keissler in Vienna, S. u. 1185. The specimen is well preserved, and in color, general appearance, and measurements agrees with our collection. Moreover, our species checks well with Marchal's (1891) rather full original report, and with the description and figures of *Sphaeronaemella fimicola* by Massee and Salmon (1902). These authors repeatedly recovered their species from dung of rabbit, fox, deer, and dog. As a check again, our

¹ Grateful acknowledgment is made of the generous assistance given by Dr. C. L. Shear, Dr. S. F. Ashby, Dr. W. Romeyn, Dr. Walter Snell, Dr. Fred J. Seaver, Dr. John A. Stevenson and the late Dr. David Linder, in the attempt to locate type material.

species was tested on like substrate, and grew vigorously on moist sterile rabbit dung.

In the absence of type material and of a fuller description of *Sphaeronaemella carnea* our fungus becomes *Sphaeronaemella fimicola* (Marchal) emend., with the following diagnosis.²

SPHAERONAEMELLA FIMICOLA (Marchal) emend.

Sphaeria fimicola Marchal.

Sphaeronaemella fimicola Marchal. Soc. R. d. Bot. d. Belgique, Bull. 30: 143. 1891.

Sphaeronaema fimicola Jaczewski. Soc. d. nat. a. Moscou, Nouv. Mem. 15: 300. 1898.

Sphaeronaemella carnea E. & E. Jour. Myc. 5-6: 152. 1889-1891.

Fungo conidiophoro: Mycelio hyalino, pubescente; hyphis 1.2-3.5 μ diam., rare usque ad 5.6 μ ; conidiis modo *Cephalosporii* et *Verticilli* praeditis atque catenis latis; magnis sporis perdurantibus in mycelio fictis circum perithecium basi, 5.6-16 μ , glabris vel verrucosis.

Fungo ascophoro: Mycelio heterothallico. Peritheciis superficialibus vel parum immersis, semper ferme gregariis sed aliquando sparsis, sphaeroideis vel ovatis, 150-250 μ diam., mollibus, luteolis vel carnosus coloribus, semper ferme glabris; in ostiolum, subulatum cylindraceo-conicum, vertice hyalino penicillato, 700-1800 μ , longitudine productis; sporulis ellipsoideis, hyalinis, leniter curvatis vel rectis, 7-8.8 μ rare 4.4-11.4 μ longis, 2-4.5 μ latis; muco obvolutis, ad ostiolo apicem demum expulsis et globulum ovoideum albidum formantibus.

Hab.: in fimo leporis usque canis, Belgium, 1883-1885; in fimo leporis usque cervae, England, 1901; in cortico Cucurbitae, New Jersey usque Connecticut, U. S. A. 1934-1945.

Specimens of this fungus are being deposited with the New York Botanical Garden, the Farlow Herbarium, and the Cornell University Herbarium. Cultures have proven so susceptible to temperature that they could be maintained only during the five years when a low temperature incubator (free from mites) was available, and therefore cannot be distributed at the present time.

SUMMARY. A species of fungus previously described as *Sphaeronaema fimicola* is reported from cucurbits in New Jersey and Connecticut. The supposed pycnidia have proved to be ascocarps, and the species is diagnosed as *Sphaeronaemella fimicola* (Marchal) emend. This mold is heterothallic, and its characteristics in culture are described and figured.

² We are indebted to Mrs. Vivian Trombetta Walker for latinizing the species diagnosis.

DESCRIPTION OF FIGURES

FIG. 1, Mature ascocarps on ten day old agar plate. $\times 8$. 2, Detail of same ascocarps. $\times 14$. 3, Young ascocarps submerged in the same agar plate. $\times 14$. 4, Tufted mycelium of a monoascospore strain after fourteen days on a sterilized piece of gray Hubbard squash rind. \times about 4. 5, Mycelium of monoascospore strain after ten days on Hubbard squash rind agar. \times about 4. 6, Fan-shaped branches of mycelium from a monoascospore strain after four weeks of growth on zucchini squash rind agar. $\times 28$. 7, Conidial masses in the mycelium of a ten day old squash rind agar culture made from mixed ascospore inoculum. $\times 68$. 8, Base of an eight months old squash rind agar slant showing the tough moist pink under surface in the region where yeast-like cells are often abundant. $\times 1\frac{1}{2}$.

FIG. 9. Free hand drawing of tufts of conidiophores at margin of three weeks old colony on sterilized squash rind. \times about 25. 10,* Mature ascocarp from four weeks old cornmeal agar plate. The spore globule at the ostiole has dried, and slipped to one side of the beak. This spore globule is often found far down the side of the beak on an old dried ascocarp. $\times 43$. 11, Tip of beak showing setae, with conidia developing at *a*. From 10 day old squash rind culture. $\times 660$. 12, Asci from a perithecium in which were many spores already freed by the deliquescing of the thick ascus wall. $\times 660$. 13, Ascospores from globules at tip of beak; *a*, mucilaginous matrix which becomes vacuolated in a water mount, and slowly breaks up; *b*, globules of matrix adhering to spore; *c*, hypha from spore germinated in the globule. $\times 66$. 14, One of a cluster of bristle-like branches developing on the original bit of inoculum on a seven months old malt extract agar slant. $\times 480$. 15, Chlamydospores from mycelium at base of ascocarp in six weeks old squash rind agar plate, showing both rough and smooth walled cells; *a*, germinating spore. $\times 660$. 16-18, Conidia. $\times 660$. 16, From nine day old squash rind agar plate, kept very moist with water well. Budding was active, and detached buds were frequently found. 17, From submerged portion of a ten day old squash rind culture. 18, From aerial conidiophores of same culture. 19, Portion of a section through a young ascocarp, with the outer wall omitted; asci in very early stages of development are shown among the gelatinizing pseudoparenchymatous cells. Section 5μ thick; iron-alum haematoxylin. $\times 660$.

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NOTES AND BRIEF ARTICLES

HEMITRICHIA VS. HYPORHAMMA: The genus *Hemitrichia* was established by Rostafinski (Versuch 14. 1873) to include a group of species of Myxomycetes, previously included for the most part in *Trichia*, characterized by a capillitium in which the spirally-marked elaters are united into a net rather than being free as in the species retained in *Trichia*. The representative species designated were *T. clavata* Pers., which may be regarded as the type of the genus, and *T. serpula* (Scop.) Fries. Two years later (Monog. p. 261. 1875) Rostafinski arbitrarily changed the generic name to *Hemiarcyria* and the species involved were generally referred to that genus until Lister (Mycetozoa, p. 174. 1894) revived the earlier name, since which time *Hemitrichia* has been universally used.

The distinction between *Trichia* and *Hemitrichia* is somewhat artificial. Certain species of *Hemitrichia*, *H. Vesparium* for example, have relatively free elaters and some species of *Trichia* may under certain conditions develop a more or less netted capillitium, but on the whole the distinction is useful and workable.

Some years before Rostafinski published, Corda (Icones, 6: 13, pl. 2, f. 34. 1854) founded the genus *Hyporhamma* upon *Trichia reticulata* Pers. (Syn. Fung. p. 182. 1801), calling it *H. reticulatum* and making it the sole representative of his new genus. This species is universally recognized as identical with what is currently known as *Hemitrichia serpula* (Scop.) Rost. Corda's species is cited by Rostafinski (Monog. p. 266. 1875) as a synonym of his *Hemiarcyria serpula*, although the volume and date of Corda's work are incorrectly given. In his generic diagnosis, Corda stresses the repent, i.e., plasmodiocarpous, character of the fructification and the keel-like base of the peridium as well as the netted capillitium. The first character is true only of *H. serpula* and, in much less degree, of *H. Karstenii*. The keeled base of the peridium is present only in occasional specimens of *H. serpula*. Reference to the illustration shows the spirally-marked capillitium

and leaves no doubt that Corda actually had before him a specimen of *H. serpula*. But the textual comment as well as the drawing makes it clear that the keel-like base which Corda stressed was actually due to the fruiting of the plasmodiocarp along the surface of a rootlet, or, more probably, a rhizomorph of some fungus occurring superficially on the substratum, which Corda regarded as belonging to the myxomycete, stating that the keel develops from the root-like strands. He evidently regarded this character as of primary importance, since he selected a name for his genus based upon it, $\nu\pi\omicron + \rho\alpha\mu\mu\alpha$ clearly referring to the basal thread.

To accept Corda's genus would involve a new combination in every species of *Hemitrichia* recognized at the present time, including the type species, since the specific epithet adopted by Corda is untenable, and would also necessitate radical emendation of the genus, because the repent habit and the carinate base are wholly inapplicable to the great majority of the species. Under such circumstances, if it were necessary, a good case could be made for conservation of *Hemitrichia* against *Hyporhamma*. Inasmuch, however, as Corda's genus was evidently based upon two discordant elements, the plasmodiocarp of the myxomycete and the rootlet or rhizomorph on which it was fruiting, it seems permissible, despite the fact that the species described is clearly recognizable, to regard *Hyporhamma* as technically a *nomen confusum* under the provisions of Art. 64 of the International Rules and advisable to publish this justification for the retention of the current and universally recognized name *Hemitrichia*.—G. W. MARTIN, Department of Botany, University of Iowa, Iowa City.

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XL

MARCH-APRIL, 1948

No. 2

DEVELOPMENTAL STUDIES OF TWO SPECIES OF *NOWAKOWSKIELLA* SCHROETER: *N. RAMOSA* BUTLER AND *N. PROFUSA* KARLING *

JOHN MAURICE ROBERTS

(WITH 2 FIGURES)

The position of the genus *Nowakowskiella* Schroeter in the phylogenetic scheme of the lower fungi is undecided. It is usually placed among the cladochytriaceous fungi, a group which has been considered to contain possible transition forms between the monocentric chytrids and the filamentous phycomycetes with true mycelium. For the thallus of all the polycentric members of the Chytridiales which were then included in the Cladochytriaceae, Karling (1932) proposed the descriptive term "rhizomycelium." Sparrow (1943) divided all the chytrids into two parallel series: the Operculatae and the Inoperculatae, thus removing *Nowakowskiella* from the family Cladochytriaceae. Whiffen (1943) suggested that it might be better to separate *Nowakowskiella* from *Cladochytrium* on the basis of the presence of septa in the intercalary swellings, rather than on the presence of an operculum; and questioned whether the swellings in the two genera were homologous.

* A condensation of a thesis submitted to the Department of Botany and Plant Pathology, Michigan State College, in partial fulfilment of the requirements for a Ph.D. degree. Present address: Mich. Dept. of Health, Bur. of Labs., Lansing 4, Mich.

[MYCOLOGIA for January-February (40: 1-126) was issued February 18, 1948]

It is true of all the lower phycomycetes that the final decision as to the tenability of a phylogenetic scheme must await further studies of the cytology, development, and reproduction of all the basic forms, especially those which appear to represent transitions. At the present time only six species of chytrids have been fully investigated (Wager, 1899, 1913; Dangeard, 1900-1901; Karling, 1937; Hillegas, 1940; and Hanson, 1945a and b, and 1946).

This study was instituted to throw more light on these possible transition forms among the lower phycomycetes.

MATERIALS AND METHODS

Cultivation. The original collection of *N. ramosa* used in this investigation was found by Professor E. A. Bessey on grass leaves in distilled water to which had been added debris from a rain-water cistern. This species and *N. profusa* were subsequently collected in a garden pool and from the relatively still water along the edge of a river.

Cultures for study and for stock maintenance were grown on hemp achenes and cellophane in sterilized river water in petri dishes. For the study of living specimens at high magnification hanging drops were suspended from cover slips on rubber washers sealed to the slides with either petroleum jelly or waterproof cement.

It was found that sterilized river water containing cellophane as the only other source of food supported abundant development and reproduction of *N. ramosa*, whereas distilled water, physiological saline, and Knop's solution would not sustain growth of this species for more than one transfer.

N. ramosa would not parasitize seedlings of barley, corn, oats, rice, rye or wheat; nor would this species attack the contents of cereal grains or hemp achenes although it would grow well on the pericarps alone. Cotton fibers and cellophane chips were digested directly by the fungus, but chemically prepared cellulose acetate was not attacked.

On initial isolation, *N. ramosa* required a temperature between 16° and 18° C. for cultivation and had to be gradually acclimated to temperatures above 20° C. *N. profusa* grew readily at 20° to 28° C. from the beginning and was inhibited by the lower temperatures.

N. ramosa indicated an affinity for free oxygen, in that this species produced better growth and zoosporangial development at the surface of the liquid in petri dishes and could not be recovered in the living state from cultures in test-tubes. *N. profusa*, on the other hand, produced more zoosporangia appressed to the bottoms of the petri dishes and developed freely under one to two inches of water in Ehrlenmeyer flasks.

Staining. Most of the cytological data in this paper are taken from material which had been killed and fixed in Sass' modified Bouin's fluid, Belling's Navashin's fluid, Flemming's weak fluid—undiluted and one-half strength, Carnoy's fluid, and the Zirkle-Erliki fluid for mitochondria. For cytological details Tuan's smear modification of the haematoxylin method and Flemming's triple stain gave the best results. Material stained by the haematoxylin method was whole-mounted directly from the last water-rinse into glycerine jelly, thus alleviating the difficulty of matching cytological structures which is encountered in the use of serial sections. With extreme care, material stained by Flemming's method could be dehydrated by running large volumes of the solutions over the specimens on a large glass plate and then mounted in balsam.

As an aid in the observation of living specimens, a drop of one per cent aqueous Porrier's blue was allowed to diffuse into the mount from the edge of the cover slip.

Zoospores were stained by the Feulgen reaction, Gram's stain and the crystal violet method described by Cotner (1930).

Microchemical Tests. Thallus walls of both species give a positive reaction to the chitosan test as described by Johannsen (1941). Picric acid gives, as Nabel (1939) described, a yellow coloration that cannot be washed out. When 75 per cent sulfuric acid is added to material treated in this way, the entire thallus dissolves. The sporangium and apophysis walls of both these species also react slightly to chlor-iodide of zinc, indicating the presence of some cellulose. Both the chitin and the cellulose seemed to be present in the same layer in both species. An inner layer in which neither cellulose nor chitin was demonstrated to be present, appeared in the sporangial wall of *N. ramosa*, but not in *N. profusa*. There was no evidence of an evanescent inner wall of chitin such as Nabel (1939) found in *Rhizidiomyces bivellatus*. These spe-

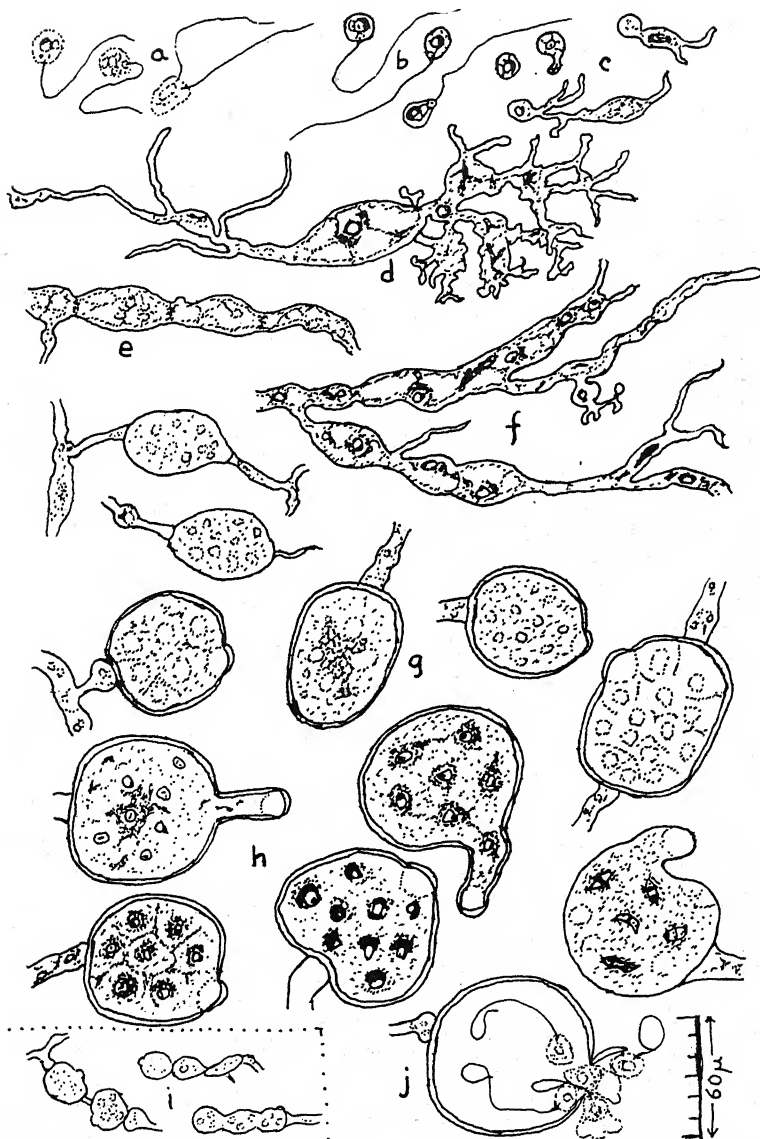
cies of *Nowakowskiella* do agree with *Rh. bivellatus* in that the walls of the filaments and rhizoids respond to tests for chitin and not for cellulose. Hillegas (1940) found the walls of the zoosporangia of *Endochytrium operculatum* to give a weak cellulose reaction, as is true with the two species being considered here, but does not mention their behavior with tests for chitin.

In the case of both *N. ramosa* and *N. profusa*, treatment with ruthenium red stains none of the fungus except the operculum. The reddish violet color acquired by this structure indicates that it is primarily of a pectic nature.

The "oil" globules and refractive matter reacted alike, whether in the zoospores, filaments, spindle-organs, or rhizoids. With Sudan IV they stained golden yellow, became light brown after standing in osmic acid, and were dissolved by acetone. These findings do not add any more information as to the composition of the "oil" in the chytrids than Karling (1937) and Hillegas (1940) obtained with *Cladochytrium replicatum* and *Endochytrium operculatum*, respectively; that is, they seem to be of a fatty nature but may be more complex. Treatment with saturated aqueous picric acid or with dilute aqueous eosin did not show any indications of proteinaceous matter with the fatty globules. This possibly was due to the immiscibility of the water-dissolved and the fatty matter. With picric acid the nuclear caps and cytoplasmic strands stain a bright greenish yellow.

DEVELOPMENT OF *N. RAMOSA*

Structure of the Zoospore. The typically single refractive globules seen in the living zoospore of *N. ramosa* are anterior or lateral, never posterior, to the centrally-located nucleus, a condition similar to that in *Solutoparies Pythii* (Whiffen, 1942). Atypical spores may exist containing two or three globules, as described for *N. elongata* by Karling (1944a) and for *Sporophlyctis rostrata* by San-Chium Sen (1944). Variability as to the refractive globule in the genus *Nowakowskiella* is common. Among the other species, *N. elegans*, *N. hemisphaerospora*, and *N. delica* (Matthews, 1928; Shanor, 1942a; and Whiffen, 1943) have been described as having a single spherical globule; *N. elongata* (Karling, 1944a) usually has a single globule but may have two. The refractive ma-

FIG. 1. *Nowakowskiella ramosa*.

terial in *N. macrospora* (Karling, 1945a) is composed of a disc-shaped globule and numerous minute granules; and *N. granulata* (Karling, 1944a) is characterized by the presence of numerous golden brown granules.

Also visible in the living spore is a minute granule, the blepharoplast, in a small vacuole posterior to the nucleus. From this granule the single flagellum extends into the surrounding medium. In the abnormal bi-, tri-, or quadriflagellate spores, which occur in *N. ramosa*, there is a blepharoplast for each flagellum. These may be located in the same or in different vacuoles. Although the multiflagellate spores may have more than one refractive globule, they contain only one nucleus. The biflagellate-uninucleate condition of *Nowakowskiella* sp. spores has also been reported by Ellison (1945). The biflagellate spores are usually somewhat larger than the uniflagellate ($10.6\text{--}21.3\ \mu$ as compared to $4.8\text{--}9.7\ \mu$), and are seldom the same size. Since fusion of two spores has never been observed and since tri- and quadriflagellate forms are found, it is believed that these multiflagellate spores are not evidence of sexuality in this species. The author prefers to believe for the present that this condition may be due to improper cleavage as reported by Cotner (1930) for *Blastocladia* and by other workers for other chytrids. Evidence in favor of the uneven cleavage theory is given by the presence of nonflagellate spores consisting of little more than a nucleus in the same slides as the multiflagellate spores. Further studies may throw more light on this strange attachment of the flagellum of one spore to the nucleus of another.

Whatever the reason for the alignment of more than one flagellum with a single nucleus, it appears to be evidence that at least the initials of these appendages are laid down between the times of division of the sporangial plasma into the spore initials by furrowing and the final separation of the mature spores preceding their escape. The flagella are evident immediately on those spores left in the zoosporangium after the first mass has escaped. Their presence was also observed by Karling (1937), Hillegas (1940), and Couch (1945) and is considered to support the belief that the flagellum is formed while the spore is still in the sporangium. This time of formation is also indicated in that spores attempting

to separate from the mass at the orifice may be observed held by a fully formed flagellum caught in the exit papilla by another spore. Some of these caught flagella have loops or vesicles at the ends. When the spore has freed itself, the loop is no longer evident. Hillegas (1940) described similar loops on the flagella of immature spores of *Endochytrium operculatum*; Berdan (1941b) found loops in various positions on the flagella of *Catenochytridium carolinianum* zoospores; and Ajello (1942) reported them on the flagella of occasional zoospores of *Polychytrium*. These looped flagella are probably the results of developmental irregularities and not homologous to the knobbed modification of the whip-lash flagella found by Ellison (1945) in some of the Mycetozoa. Karling (1945b) and Hanson (1945a) have recently described loop formation in the absorption of the flagella when the spores become sessile.

Usually there is a second vacuole, other than that containing the blepharoplast in the spores of *N. ramosa*. Its position is lateral or anterior, frequently opposite the globule in relation to the nucleus. Under unfavorable conditions, such as too long exposure to distilled water, this vacuole may swell until the spore has become distended into little more than a tonoplast about a huge (in relation to the usual spore size) vacuole. When the spore is in this distended condition the nucleus and nuclear cap are compressed into the posterior end near the point of insertion of the flagellum and motility by flagellar or amoeboid action ceases.

As is becoming increasingly evident among the chytrids, the most conspicuous structure in a stained zoospore of *N. ramosa* is a crescent-shaped nuclear cap, surrounding a third or more of the centrally-located nucleus. The finding of nuclear caps in the zoospores of the chytrids prompted Ajello (1942) to suggest that these extra-nuclear structures are not as important phylogenetically as once thought. In the spores of this species the nuclear caps may vary from more or less thin crescents to cups or spheres. The crescent-form and the cup-form are usually tilted so that the cap is oriented with the thicker part to one side as may occur in the spores of *Catenochytridium laterale* (Hanson, 1946) and not directly anterior to the center. The spherical form appears to be a hollow ball which is filled with the nucleus.

Lateral to the nucleus is a large clear space, which appears to be left by the dissolution of the refractive globule of the living spore. If this space does represent the position of the globule, it indicates a fixed position for that structure relative to the nucleus and nuclear cap. For *Endochytrium operculatum*, Hillegas (1940) considers a similar clear space to be the nucleus.

Most of the fixed and stained spores exhibit a distinct blepharoplast and rhizoplast associated with the flagellum, such as have been described in chytrid zoospores before (Berdan, 1941; Karling, 1937; and Hillegas, 1940). Ellison (1945) uses the presence of these structures for designating that the swimming organelle of the phycomycete zoospore is a true flagellum. Usually the stained spore lies so that the thin rhizoplast can be seen passing diagonally from the blepharoplast to a point of attachment at the nucleus in the vicinity of one of the pointed horns of the cap. Karling (1942) described the nucleus tapering to the point of attachment of the flagellum in the zoospore of *Septochytrium macrosporum*, in a similar manner to the sub-triangular nuclei in the zoospores of *Blastocladiella simplex* and *Blastocladia* (Matthews, 1937; Cotner, 1930). Either a similar tapering structure or two granular cytoplasmic strands (one or both functioning as a rhizoplast) extend from an apex at the blepharoplast to the posterior side of the tilted nuclear cap in some spores of the present species. Only one of the strands joins the central structures at the tip of a horn of the cap. If this really be a conical extension of the nucleus, a second opening in the cap must be presupposed; if it be strands, the structure is like that found in *Monoblepharella Taylorii* (Springer, 1945) and occasionally in *Blastocladia* (Cotner, 1930).

When the spore becomes sessile (between four and twelve hours after release from the sporangium) the well-defined nuclear cap disappears, in contrast to the occurrence in *Cladochytrium replicatum*, *Endochytrium operculatum*, and *Polychytrium stromaphilum* in which the cap may persist during the early stages of germination (Karling, 1937; Hillegas, 1940; Ajello, 1942). In place of the cap there is a deposit of large granules around the outside of the nuclear membrane. Larger granules also become apparent in the cytoplasm, radiating in elongated masses from the nuclear to the plasma membrane, lining the inside periphery of the cell, and sur-

rounding small vacuoles. Examination of the spores which appear to be in transitional stages in the disappearance of the cap indicates that this structure is the source of the majority of these large granules. The clear space which is adjacent to the nucleus and the nuclear cap in the swimming spore may or may not be present or may be partially filled with granules. Here again the behavior of this clear space in the stained spore parallels that of the globule which becomes smaller and disappears in the living. The nucleus contains a central nucleolus and a fine reticulum.

Germination of the Spore. Between six and ten hours after the zoospore of *N. ramosa* has become sessile, it puts forth a single germ-tube which usually sends off one branch before it has grown longer than the diameter of the spore. Although the germ-tube may branch two or three times before there are any swellings formed, the most common development results in a primary swelling, which may be homologous to the primary spindle-organ in *Cladochytrium replicatum* (Karling, 1937), before the second dichotomy occurs. A very young thallus with one branch and one swelling resembles the early stages of the type 3 formation of the apophysis in *Catenochytridium laterale* (Hanson, 1946). The nucleus may remain in the spore case until the primary swelling has formed or may migrate into the unswollen tube which enlarges around it, as Hillegas (1940) has described for *Endochytrium operculatum*. If the nucleus remains in the spore case, it may divide and the daughter nuclei migrate to the primary swelling, one at a time, thus differing in this respect from *Cladochytrium replicatum*, *Endochytrium operculatum*, and *N. hemisphaerospora* (Karling, 1937; Hillegas, 1940; Shanor, 1942a). In order to pass through the narrow filament from the spore-case to the enlargement, the nucleus becomes elongated and narrow, in the same manner as Karling (1937) reported for *Cladochytrium replicatum*.

After the primary swelling has formed there is no further growth originating from the spore, even though the spore case may persist throughout the life of the thallus. The persistence of the spore case may be ontogenetic evidence of rhizidiaceous ancestry. Unlike *Cladochytrium hyalinum* (Berdan, 1941a) the spore case is not emptied of cytoplasm before the formation of the primary swelling whether the nucleus has migrated into the tube or not.

The branches of the germ-tube may be so close together that there appear to be two or three germ-tubes from a single spore under low magnification. However, this is a false impression; and the fact that a single germ-tube is formed seems to be important in the light of Couch's (1945) having used the two germ-tubes in *Catenaria* as one of the points for transferring that genus from the *Chytridiales* to the *Blastocladales*.

The Vegetative Portion of the Thallus. After the primary swelling and its rhizoidal offshoots have become established, the thallus continues to grow, branching dichotomously and producing additional nucleate swellings. No cross walls are formed in the extension of the thallus. It differs in this respect from *Cladochytrium replicatum* and *C. tenue*, *Catenomyces*, and *Catenaria*. For one or two days in *N. ramosa* most of the growth consists of the establishment of spherical, fusiform, and irregular swellings appressed to and imbedded in the substratum. In the mature thallus numerous much-branched rhizoids arise from the thallus and from the isthmuses between. When the fungus is grown in grass leaves, the swellings may occupy part or all of the cavity of the host cell; and the rhizoids dissolve their way into and ramify in the cell walls. On cellophane chips, the swellings are appressed to the surface; and the rhizoids lie in channels of fluid digested into the solid substratum. The rhizoids are short and may be expanded in spots, especially at the points of much-branching, into bladder-like formations with thin walls and large vacuoles. Another polycentric operculate chytrid forming thick, short rhizoids in cellophane is *Catenomyces persicinus* (Hanson, 1945a). Some of the bladders are so great in diameter in places of pronounced branching that they can be distinguished from the centers of development only by the lack of nuclei, and some by the thinner wall. Except for *Nowakowskiella* not containing cross-walls this network of rhizoids and swellings resembles the thallus of *Megachytrium Westonii* (Sparrow, 1933) more closely than it does any of the other polycentric chytrids.

The most pronounced inclusions in the living protoplasm of the vegetative system are the refractive globules, which resemble the globules of the zoospores, except for the former's great variation in size. The presence of similar refractive globules in the intra-

matrical portion of the thallus has been reported before for *N. profusa* (Karling, 1944), as well as for *Cladochytrium replicatum* (Karling, 1937), *Catenomyces persicinus* (Hanson, 1945a), *Cladochytrium hyalinum*, and *Catenochytridium carolinianum* (Berdan, 1941a and b). Few scattered minute granules are present in the rhizoids which have digested their way into the substratum; but wherever a swelling occurs, there is an accumulation of globules, which may fill the swelling as a single mass in a mature thallus.

The hyaline cytoplasm appears to fill the absorptive rhizoids and to surround and divide diagonally large vacuoles in the swellings. The vacuolate nature of the cytoplasm of the vegetative portions of *Cladochytrium replicatum*, *Endochytrium operculatum*, and *Catenaria* has been described by Karling (1937), Hillegas (1940), and Couch (1945), respectively. The globules are in intravacuolar groups, held in the cytoplasmic strands.

Fixing and staining procedures dissolve out the refractive matter and accentuate the structure of the cytoplasm and the nuclei. If osmic acid is used in a fixative and is not bleached out, the place occupied by the refractive matter contains a brownish, amorphous mass. Otherwise, the spaces occupied by the globules are clear except for loose clumps of densely-staining granules. Similar granules are found scattered through the cytoplasmic strands. The number of granules present in *N. ramosa* and *N. profusa* seems to be greater than that for *Cladochytrium replicatum* (Karling, 1937). In many instances the nuclei are surrounded by masses of these granules. In their affinity for the nucleus these granules resemble those which make up the nuclear cap of the zoospore.

In older thalli strictures in the isthmuses between the swellings may be filled with cytoplasm, often of a denser appearance than that in the swellings, forming a type of pseudoseptum. Similar bands of material were found in the rhizoids of *Septochytrium variabile* by Berdan (1942).

THE REPRODUCTIVE PORTION OF THE THALLUS

Flexuous Filaments. Arising from the sides or ends of the vegetative swellings or from the isthmuses between are long,

flexuous, usually extramatrical filaments which branch by repeated dichotomies. These filaments may be isodiametric throughout their entire length or may vary considerably in diameter. Elongate fusiform swellings, which are smooth in outline, may usually be found regularly in the proximal one-third of each filament. Rarely were secondarily developed, typical absorptive-vegetative centers found arising from these swellings. Distal to this area, the swellings are irregular in shape and length: being fusiform, spherical, or even triangular at points of branching, to mere undulations in the otherwise parallel filament walls. The narrower portions range in diameter from 1.5 to $2.5\ \mu$ in the more distal segments, while the swellings in these segments are only 2.0 to $4.0\ \mu$. The length of the narrower portions is 4.0 to $40.0\ \mu$, as opposed to 11.5 to $30.0\ \mu$ for the swellings. In this area it is difficult to draw a clear-cut differentiation as to what constitutes a swelling and what an isthmus. If one overlooks the small differences in diameter, it is possible to consider segments as long as $300\ \mu$ as lacking true swellings. The swellings and isthmuses in the proximal portion are more easily differentiated, since their sizes are $2.5\text{--}5.0\ \mu \times 6.0\text{--}11.0\ \mu$ and $1.5\text{--}2.5\ \mu \times 4.0\text{--}30.0\ \mu$, respectively. It is not uncommon to find one of these flexuous filaments almost twice as wide in the distal portion as in the proximal. Lateral branches may form at almost right angles and remain only 1.0 to $1.5\ \mu$ in diameter.

Internally these filaments may resemble true tubular coenocytes. The cytoplasm in the younger thalli contains many deeply-staining granules and small refractive globules, and is netted with small vacuoles. In older thalli the vacuoles are longer, surrounded and crossed by thin strands of minutely-granular cytoplasm. As in the vegetative system, pseudo-septa of thick cytoplasm may be found in points of constriction.

The presence of nuclei in these filaments is variable. They are usually absent in the narrow lateral branches, and are usually present in the swellings. Up to six nuclei have been found in a single enlargement. Mature thalli may bear irregularly swollen filaments or filaments lacking swellings in which rounded nuclei ($2.0\ \mu$ in diameter) are scattered throughout their lengths. As many as fourteen nuclei have been observed evenly distributed in a fila-

ment of *N. ramosa* 485 μ long from its origin to the cross-wall separating the zoosporangium from the rest of the tube. The regular distribution and the rounded shapes and lateral position of the nucleoli in the nuclei make it appear that these structures are fixed in their places, rather than passing through the filaments. Karling (1937) described the moving nucleus in *C. replicatum* as elongate and densely-staining. Hillegas (1940) reported that the nuclei of *Endochytrium operculatum* elongate only when passing through a constriction in a rhizoid. Since the author has observed nuclei scattered in the majority of the filaments which were alive just prior to the time of killing and fixing, it is his opinion that these structures are typically nucleate in both *N. ramosa* and *N. profusa*.

Formation of the Zoosporangium. Each of the flexous filaments described above may bear zoosporangia which are terminal, intercalary, or on short lateral branches. Most of the zoosporangia are extramatrix, rarely intramatrix. The author has never observed a typical vegetative swelling producing zoospores. In this way these species of *Nowakowskiella* differ from *Cladochytrium replicatum* (Karling, 1937) and *Megachytrium Westonii* (Sparrow, 1933). The thallus of *Physocladia* (Sparrow, 1932) exhibits a similar specialization. The differentiation of the thalli into vegetative and reproductive portions is pronounced in *N. ramosa* and *N. profusa*.

On actively-growing thalli of *N. ramosa*, the continuation of the filament distal to the zoosporangium is usually a fine thread which shrivels as the sporangium matures. In the few instances where this thread has persisted, it has been found to be anastomosed with another, larger flexuous filament. Most often a filament of *N. ramosa* branches near its end, and each of the equally long branches is terminated by a zoosporangium. Examination of thalli bearing predominantly zoosporangia gives the impression that these reproductive bodies are terminal in *N. ramosa*. The filament below the sporangium of *N. ramosa* is swollen into an infundibuliform sub-sporangial swelling.

Elongate exit tubes develop on those sporangia that are intramatrix or surrounded by a zooglycal slime, and do not extend beyond the outer edge of the enveloping material. A zoosporangium

of *N. ramosa* may bear one to three branched exit tubes or papillae, only one of which is functional.

At their inception the swellings which develop into zoosporangia are usually intercalary. If the enlargement begins near the tip of the filament, the tip may be included in the rounding-out of the sporangial rudiment; or the tip may shrivel, as described above. The swellings are usually fusiform-elongate at first, resembling those in the lower third of the flexuous filaments. They increase in circumference more rapidly than they do in length until they have become spherical in shape. Deviations from this typical spherical shape are pyriform, ovate, obtusely-branched, obtusely-triangular, and hourglass-shaped. When the rudiment is about one-half its mature size a cross-wall forms, dividing the swelling into two unequal parts. This time of delimiting the incipient zoosporangium differs from that of *N. delica* in which the cross-septum is formed when the swelling has reached mature size (Whiffen, 1943). The smaller portion of the swelling continues to enlarge into an evenly-shaped funnel form in *N. ramosa* so there is no constriction at the cross wall.

When the incipient sporangium has reached mature size, the wall and cross-septum increase in thickness. At one point, lateral, sub-apical, sub-basal, or rarely apical, the sporangium wall forms a disc-shaped area which is thicker and more refractile than the rest. Around the circumference of this disc, the wall is very thin, seeming to consist of little more than the external layer of the immature thin-walled swelling. If exit tubes or papillae have formed, this disc is at the tip of a tube or a papilla; otherwise it is flush with the surface of the wall. This disc becomes the pectinaceous operculum which bulges as a slight convexity on the mature sporangium wall.

Zoosporogenesis. Appearance of Living Material. The behavior of the protoplasm in developing sporangia of *N. ramosa* is essentially the same as that described for other chytrids (Berdan, 1941a and b and 1942; Couch, 1945; Hanson, 1945a and b; Hillegas, 1940; Karling, 1937, 1944a and b, and 1945a, b, and c; Whiffen, 1942 and 1943). Because of this similarity, the author will refer to the observations of other workers only where differences in structure and behavior of the organisms seem significant.

As the swelling which develops into a zoosporangium grows, the refractive content of the otherwise hyaline cytoplasm begins to increase in the enlargement. At the time of cross-wall formation the refractive matter appears to be concentrated in the center of the swelling. At this time several large vacuoles may be observed in the incipient sporangium, and a few large globules are present. Unlike the condition in the monocentric chytrids, the filaments supporting the sporangia of this species are not devoid of cytoplasm when cross-walls are formed. Shortly after the formation of the cross-wall the large globules in the growing sporangium increase in size and number. Later these globules again disperse, and the evenly granular appearance returns to the cytoplasm. During this stage of evenly granular appearance, the sporangium attains its mature size. Also during most of the granular stage the vacuoles are not as evident as before. Soon, however, elongate, narrow vacuoles can be seen forming in the cytoplasm, dividing it into irregularly-shaped masses. This cleavage progresses primarily from the outer edge toward the center; but additional vacuoles may be found arising independently of the primary furrows, cutting the cytoplasm from the inside out, radially, or tangentially. In each of the masses so formed the minute oil droplets coalesce into a single, or at the most two, globules. Except for the refractive globules, the cytoplasm acquires a homogeneous appearance and swells until the vacuolar divisions are obscured. In this stage the cytoplasm completely fills the sporangial swelling, except for a clear space which has formed below the operculum. Because of its spherical shape, maintained at the expense of the rest of the contents, this space appears to be filled with cytoplasm such as in the body of the sporangium up to the clear space which is just below the operculum at the tip of the tube.

Appearance of Stained Material. In the stained material the development of the sporangium has been followed by the study of cells of different sizes, using the size as an indication of relative maturity. This method seemed practical because the size of the swelling and the number of nuclei ordinarily paralleled each other.

When the small intercalary swelling first appears it is filled with a granular cytoplasm and exhibits one nucleus about 2μ in diameter. The deeply-staining granules are small, with larger granules

massing in streaks through the cytoplasm. Often these streaks are associated at one end with the nucleus, an observation similar to that of Karling (1937) in the newly nucleated spindle-organ of *C. replicatum*. The author's observations on *N. ramosa* do not affect the theory that the strand represents the line of passage of the nucleus. As the swelling enlarges these strands disappear and the granules become evenly distributed throughout the sporangium rudiment. The tapering portion of the enlargement which is to become the subsporangial swelling is filled with cytoplasm and may contain one or more nuclei like those found in the sporangium rudiment. Several large nuclei are found in the rudiment before it has enlarged very much. In a few preparations the first nucleus in the rudiment has been observed to be dividing; but there was no proof discovered that all the nuclei in it came from continued divisions of this single nucleus or that some of them might not have migrated into the swelling from the proximal or distal filaments. The solution of this question is further confused since there are several nuclei in the swelling before the septum cuts it off from the rest of the filament.

As the swelling continues to enlarge into a spherical form and more nuclei are found in it, the deeply-staining granules aggregate loosely in the center, leaving the remainder of the cytoplasm less densely granular. When the rounded form of the swelling is established, the adjacent portion of the proximal filament has swollen to be infundibuliform. The distal continuation of the filament begins to shrivel back to the rounded swelling in those sporangia which appear terminal at maturity although intercalary in origin. If the distal filament persists, it may be swollen or not. The flexuous filaments on which the sporangia are borne are usually almost devoid of nuclei and highly vacuolate by the time the sporangium is mature. The vacuolate nature of the cytoplasm is less prominent nearer the sporangium, and nuclei may be present in the swollen filament immediately below the sporangium. This is also true of the distal continuation of the filament if the sporangium is truly intercalary, or if this portion has anastomosed with another filament as described above.

The granular mass in the center of the sporangium disintegrates as a densely granular reticulum spreads throughout the

swelling. The nuclei become aligned on strands of this reticulum in such a way that observation of subsequent nuclear divisions is made difficult. Some strands of reticulum extend down to where the tapering of the swelling begins; and at this place, a thick plate of granules extends across the swelling. As this plate becomes more apparent, the outer wall of the sporangium begins to thicken, and the papillar swelling begins to grow through any material present that will inhibit the free escape of the zoospores. As the plate which will form the cross-septum becomes more compact and densely granular, it bulges up from the filament into the sporangium, as though the pressure exerted in the filament were greater than that in the sporangium itself. The granules forming the reticulum again become dispersed, and the cytoplasm is again uniformly granular. The granules present at this time are minute and deeply-staining. The area immediately below the exit papilla is devoid of stainable cytoplasm at this stage.

The peripheral wall of the sporangium becomes thick and deeply-staining, and the arched operculum is evident as a lightly-stained dome which is thick in the center and tapers off toward the edges. At the point where the operculum is attached to the rest of the wall, the wall is not thickened but has remained a thin ring. By the time the operculum is fully developed the cross-septum between the sporangium and the filament has the same structure as the rest of the sporangium wall.

After the walls are completely formed, the cytoplasm begins to be furrowed at the periphery by the elongated vacuoles mentioned above. This furrowing by vacuoles progresses centripetally and laterally until each nucleus has a mass of cytoplasm cut out around it. The rest of the cytoplasm in each of these areas becomes clearer as the deeply-staining granular material collects around each of the nuclei. The granular mass around the nucleus consolidates and takes the form of a loosely-packed, over-sized nuclear cap. After the cytoplasm swells so as to obscure the divisions and fills the sporangium case, the nuclear caps become more compact and more evenly and deeply-staining. The nucleolus must function independently of the nuclear cap, because it is evident as a small, dark body in the center of the nucleus before and after the granules begin to clump about the nucleus.

The above observations as to the formation of the nuclear cap tend to support Karling's (1937) belief that this structure arises from chromatic bodies or granules in the cytoplasm and is external to the nucleus itself. As with Karling's (1937) and Hillegas's (1940) material, *N. ramosa* demonstrates easily-stained nuclear caps after non-chondriosomal fixatives. It is of interest to note the similarity of behavior between the granules which form the nuclear cap and the refractive globules in their aggregation and dispersion in the incipient sporangium. The presence of similar deeply-staining granules in the vegetative portion of the thallus which in the living state contains a great deal of "oil globule" material indicates that the dispersed cap material and the "oil" may be closely associated throughout the life of the thallus. It may be that the granular material is carried dispersed in a lipoidal medium; and extrudes the lipid as the "oil globule" of the zoospore in the process of aggregating as the compact nuclear cap. The proximity of the cap and the globule in the swimming spore appears to support this belief. Since both the globule and the cap lose their distinctive identities before and during the germination of the spore, the suggestions by Karling and Hillegas that the cap is food-material which is used up in the process seem to apply to *N. ramosa*.

Although the present author has not attained the perfection of staining the dividing nuclei demonstrated by the investigators at Columbia University (Karling, Hillegas, and Hanson), he was able to observe definite mitotic figures in both species of *Nowakowskiella* studied. The nuclear behavior differs in these species from that in *C. replicatum*, *Endochytrium operculatum* and *Catenochytridium laterale* (Karling, 1937; Hillegas, 1940; Hanson, 1946) in that the nucleus does not increase appreciably in size before division and divides repeatedly during the enlargement of the incipient sporangium before and after the formation of the cross-wall. Although the division of all the nuclei in a single sporangium occurs simultaneously, it is not absolutely synchronous, since nuclei in various stages of division may be observed in a single swelling.

Zoospore Escape. For an indefinite time after maturation of the zoosporangium and zoosporogenesis, no further visible change

takes place in the sporangium. When conditions are naturally or artificially conducive to zoospore discharge, the entire contents begin to revolve in an undivided mass within the zoosporangium wall. While the cytoplasm is in motion, the operculum is forced clear off or is thrown back as on a hinge, and the subopercular material is expelled. After the operculum dehisces, the cleavage lines in the sporangium again become distinct and the irregularly shaped individual spores are evident.

The zoospores escape as individuals. The first to pass through the orifice flow through rapidly, apparently forced from behind by those adjacent to them. A mass of zoospores may form at the outside of the exit papilla, but the individuality of these spores is evident both before and after their escape. The present author would like to suggest that the spores escape into a mass, rather than *en masse*. In both species of *Nowakowskiella* considered in this paper, a single spore must be elongated and distorted to pass through the exit orifice; since the opening is but one-half the diameter of the typical spherical zoospore and the surrounding sporangium wall is rigid compared to the naked protoplast of the spore. No vesicle or any other confining material was found enclosing the mass of spores at the tip of the papilla. Whenever the outline of such a structure was suspected, continued observation showed the line to be the entangled flagellum of one of the spores in the mass. The existence of the mass is short-lived, only long enough for the spores to round up, free their flagella, and swim away.

The number of zoospores formed per sporangium varies. The zoosporangia producing the fewest spores have been observed in cultures of *N. ramosa* when very small sporangia on short filaments were observed early in the life of the thallus and contained only four or eight spores. The average number of spores for a normal zoosporangium in this species has been determined as about thirty-six.

Fusion of Cells and the Resting Bodies of N. ramosa. In many of the older cultures resting bodies were formed developing from a pseudoparenchyma as described by Butler (1907). These cells have yellowish walls of considerable thickness, which are smooth in most cases. However, it is not uncommon to find resting

bodies with the outer wall corrugated and with the inner wall exhibiting striations.

When the culture or the strain in culture has aged considerably (two or three months, for transfers were ordinarily made every two or three weeks), instead of being so fruitful in producing zoosporangia, short swollen lateral outgrowths form from the flexuous filaments.

These outgrowths may be merely irregularly-shaped enlargements which are formed by the filament swelling on one side only, or they may be short side branches consisting of thin isthmuses terminated by club-shaped tips. Two of these swellings come into contact and become superimposed upon each other. The author had difficulty in determining whether these cells actually fuse or if their cell walls merely adhered to each other. Whichever occurs, they are found in this appressed condition often enough to indicate that they are fastened together in some manner. Both cells retain their rounded shape, being flattened only where they are in contact. The cytoplasm in these cells is minutely granular, and each of them contains a single nucleus. In some cases the resultant structures are pretty definitely single cells with two rounded hemispheres, produced by the fusion of the two swellings. Whatever the case may be, nuclear fusion has not been observed. Karling (1944a) has described the formation of pseudoparenchyma from single swellings and single lateral branches, as well as from the fused tips of branches in strains of *N. ramosa* collected in Brazil. This "pooling" of the contents of two cells from different filaments may be homologous with the behavior of the two cell-potentials from which arise the resting spores of *N. hemisphaerospora* (Shanor, 1942a). In the Brazilian strains in which the pseudoparenchyma is produced without "fusion" of cells, but from a single cell, it is possible that a procedure such as has been reported for *N. hemisphaerospora* occurs during the formation of the pseudoparenchyma. The first change that occurs in the "fused" cells is the formation of large vacuoles with the tonoplasts extending across the cells so as to separate them into four to eight clear areas. A cell wall is laid down where each line of cytoplasm is present, in such a way that each of the "fused" cells is divided into as many daughter cells as there were vacuoles. Not all of these daughter

cells contain nuclei. Those that do not are either continuous with the mother filament or give rise to apophyses as described below.

The nucleate cells in the pseudoparenchyma may produce short off-shoots which have clavate tips. These tips are filled with a granular cytoplasm as they enlarge. After they have attained a more or less spherical shape, the nucleus from the pseudoparenchyma cell migrates into the apical swelling, and a plate of cytoplasm forms across the base of the rounded resting-cell initial. The nucleus takes a central position in the cell surrounded by a loosely-packed mass of granules. From this center, strands of granules radiate to the periphery of the cell. The presence of similar chromatic granules has been reported for *Polyphagus Euglenae*, *C. replicatum*, and *Endochytrium operculatum* (Wager, 1913; Karling, 1937; Hillegas, 1940). The nucleus in this resting cell was observed not to divide but to remain single and centrally placed. The uninucleate condition until mature size is attained has also been observed in *C. replicatum* and *Endochytrium operculatum* (Karling, 1937 and Hillegas, 1940).

The living cell shows a dispersion of oil droplets evenly throughout the cell and then a re-coalescing of the droplets into about eight large globules. Stained material shows the deeply-staining granules to follow the same procedure. The cytoplasm remains reticulate. Although the peripheral wall is thickened slightly by the time the cross-septum is completed, after the cell has become more stabilized as to its internal structure a striated and thicker inner wall is laid down interior to the original.

These resting bodies have not been observed to germinate in the present strain, but Karling (1944a) observed them to produce zoospores directly or to function as prosperangia.

DEVELOPMENT OF *N. PROFUSA*

N. profusa differs from the other species of *Nowakowskiella* in having a coarse, profuse rhizomycelium with few or no well-defined spindle-organs, smaller spore size, and yellow-brown resting spores (Karling, 1941). The present strain fits Karling's description with the exception that no resting spores were observed.

In most instances the development of this species is the same as has been described for *N. ramosa* in the preceding section. The

first differences between the two species to be obvious are that the flexuous filaments of *N. profusa* are almost isodiametric for their full length and that the asexual cycle of *N. profusa* takes longer for completion than does that of *N. ramosa*. Other less obvious differences are noted below.

The refractive globule is usually lateral and posterior to the nucleus; and smaller refractive globules in the posterior area may be present as Karling (1945a) described for *N. macrospora*. Unlike the globule in *N. ramosa* (Butler, 1907) and in *Endochytrium operculatum* (Hillegas, 1940), that in *N. profusa* is not fluid nor does it change shape. When the spore is escaping from the zoosporangium or is resorting to amoeboid motion, as it may do repeatedly, the globule remains in its posterior position and keeps its spherical shape. Under such circumstances, hyaline pseudopodia extend anteriorly and laterally from the spore, while the granular and refractive material of the spore remains faintly delimited as an internal spherical mass.

The vacuole, other than that containing the blepharoplast, most often is about the same size as the refractive globule or slightly smaller. The deeply-staining portion of the nuclear cap may be less dense than that in *N. ramosa*. Frequently an enclosed clear sphere containing a nucleolus-like structure, all surrounded by cap, is exhibited in such cases. This is thought to be further evidence that the nucleus is centrally located within the cap.

When the zoospores are escaping, the flagella have been observed to be entangled so frequently that the present author feels that this might be the mechanism explaining the massing of spores on discharge. The average number of spores in a typical sporangium is between sixty and eighty. No very small sporangia as were found in *N. ramosa* were observed in *N. profusa*.

The profusion of extramatrix growth of this species can be traced to the numerous much-branched filaments that arise from five to eighteen main "trunks" originating at a single rhizoidal system. Also, the filament distal to an intercalary zoosporangium is wide and branches one or more times before narrowing to a blunt end or being terminated by another zoosporangium. Thalli bearing predominantly mature sporangia give the impression that

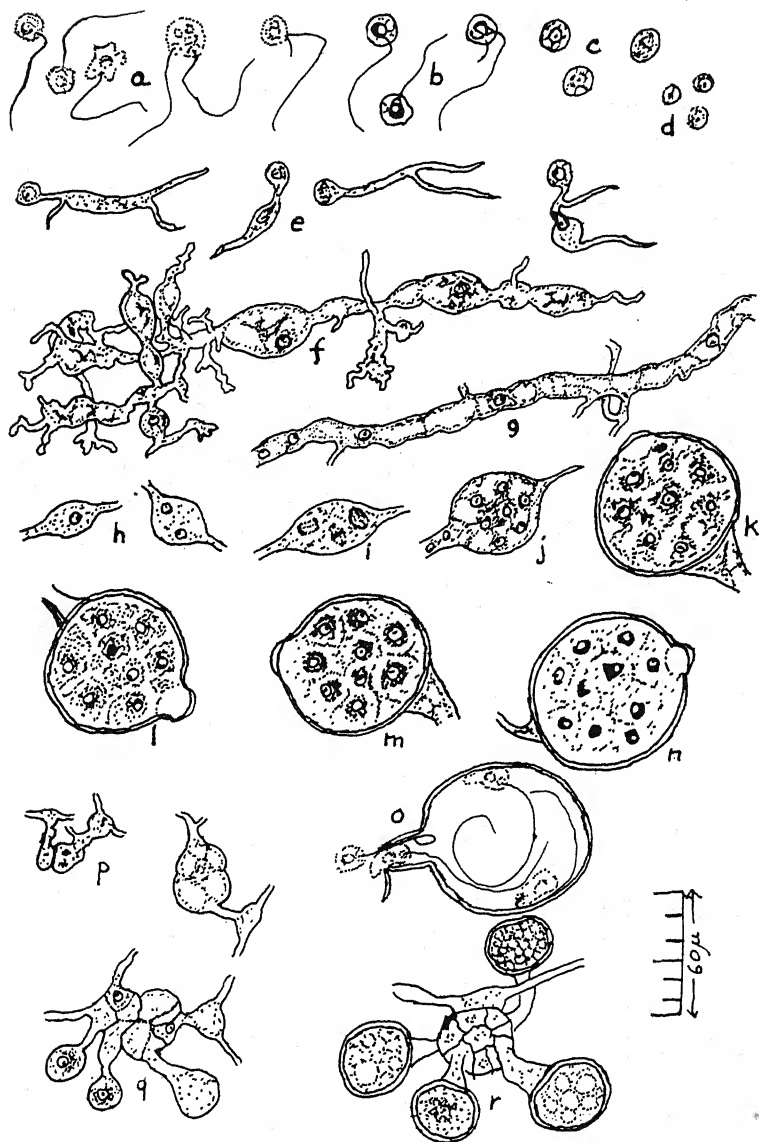
FIG. 2. *Nowakowskiella profusa*.

TABLE OF MEASUREMENTS FOR STRAINS STUDIED
(expressed in microns)

Structure	<i>N. ramosa</i>	<i>N. profusa</i>
<i>Reproductive Structures</i>		
Zoospore		
Diameter of spherical spore	4.85-9.7	3.9-4.7
Dimensions of amoeboid spore	5.82-7.7 × 9.7-15.5	3.5-7.7 × 3.5-4.5
Length of flagellum	24-35	20-30
Diameter of nuclear cap	2.0-2.3	1.5-2.0
Diameter of oil globule	2.5-3.0	1.0-2.0
Zoosporangium		
Diameter of spherical sporangium	27.16-31.37	17.0-40.0
Dimensions of sporangium other than spherical	35.9-56.3 × 37.0-77.6	11-20 × 40-50
Length of papilla		
Usual form	2.0-15.0	4.0-6.0
Long variety	50.0-80.0	up to 30
Diameter of papilla	4.5-10.0	4.0-8.0
Diameter of operculum	2.91-8.7	3.0-4.0
Thickness of operculum	0.9-1.9	0.6-1.0
Diameter of sub-opercular space	1.8-5.0	1.5-6.0
Thickness of wall	0.6-1.3	0.6-1.0
Diameter of nucleus	1.5-2.0	1.5-2.0
Subsporangial Swelling		
Diameter	3.9-13.6	0 or 2.0-6.5
Length	6.8-15.5	7.0-16.0
Thickness of wall	0.9	0.5-0.7
Diameter of subtending filament	2.0-5.0	1.5-4.0
Resting Cell		
Diameter	7.5-15.0	—
Thickness of wall	0.5-0.6	—
Diameter of nucleus	2.0-5.0	—
Flexuous Filaments		
Swelling		
Diameter of cell		
Near vegetative center	7.0-10.0	2.5-5.0
In distal portion	2.8-7.0	2.0-2.5
Length of cell		
Near vegetative center	7.0-12.0	6.0-11.0
In distal portion	11.0-35.0	11.5-30.0
Thickness of wall	0.2 or less	0.2 or less
Diameter of nucleus	1.5-2.0	1.5-2.0
Isthmus		
Length		
Near vegetative center	2.0-25.0	4.0-30.0
In distal portion	5.0-60.0	4.0-40.0
Diameter		
Near vegetative center	1.7-4.0	1.5-2.5
In distal portion	1.5-5.0	2.0-4.0
Thickness of wall	0.9	0.5-0.7
Diameter of nucleus	1.5-2.5	1.5-2.0
<i>Vegetative Center</i>		
Swelling		
Diameter of cell	4.0-20.0	3.0-12.0
Length of cell	4.0-30.0	4.0-24.0
Diameter of globules	0.2-3.0	0.2-2.0
Thickness of wall	0.2 or less	0.2 or less
Diameter of nucleus	1.5-2.0	2.0

TABLE OF MEASUREMENTS FOR STRAINS STUDIED—*Continued*

Structure	<i>N. ramosa</i>	<i>N. profusa</i>
Isthmus		
Length	0.0–7.0	0.0–6.0
Diameter	0.5–1.5	0.5–2.0
Thickness of wall	0.2 or less	0.2 or less
Rhizoids		
Length		
Branched	9.0–60.0	9.0–48.0
Unbranched	2.0–30.0	2.0–20.0
Diameter		
Branched	0.5–1.5	0.5–2.0
Unbranched	0.5–0.8	0.5–1.0
<i>Extent of Vegetative Portion</i>	35×50– 35×200	40×40– 16×160
<i>Extent of Reproductive Portion</i>	up to 2 cm.	up to 2.5 mm.

these reproductive bodies are most often intercalary, although some may be formed on the tips of short lateral branches.

The present strain of *N. profusa*, as Karling's (1941) original strain, rarely has subsporangial swellings. If they do occur, they are nearly spherical and are constricted at the cross-septum between the swelling and the sporangium. It is not uncommon to find a short tenuous filament below the sporangium leading from an intercalary swelling as in *N. elongata* (Karling, 1944a). The portions of the filament above and below the cross-septum continue to enlarge, the incipient sporangium the more rapidly, in such a way as to leave the constriction between two unequal-sized spherical swellings.

The behavior of the cytoplasm in the developing zoosporangia and the spherical subsporangial swellings, if present, is very similar to that in the homologous structures in *N. ramosa*. However, the loss of nuclei and the increase in vacuolation in the filaments which support the sporangia is less pronounced in *N. profusa* than in *N. ramosa*. Possibly the presence of more intercalary swellings in the latter is a factor affecting the presence of nuclei in the non-swollen filaments.

True internal proliferation of the zoosporangium has not been observed by the author in either *N. ramosa* or *N. profusa*; but an unusual type of proliferation is common in the latter. As the culture becomes older, the swollen or non-swollen filament above and below the original sporangium may enlarge and become divided into a chain of two to eight secondary zoosporangia. If the swell-

ings form close together their adjacent walls are flattened where they meet in enlarging. Less often there is a short isthmus between two sporangia in a chain. In either case, each individual sporangium lays down a complete thickened wall. Except for the progressive formation and the double adjacent walls, these chains of sporangia resemble the multiseptate zoosporangia of *N. elongata*. After the chains of secondary sporangia form, the filaments appear emptied of nuclei and cytoplasm. These reproductive structures do not always mature and discharge their spores at the same time, even though they may be adjacent on the same filament.

DISCUSSION

The long extramatrical filaments which arise from the vegetative portion of the thallus, often in great enough numbers to obscure it, are referred to here, collectively, as the "reproductive portion" because each of these filaments may potentially bear intercalary or terminal zoosporangia or, in *N. ramosa*, resting cells. In both the *N. ramosa* and *N. profusa* strains studied the "vegetative" and "reproductive" portions of the thallus are distinct from one another in external and internal structure. The vegetative portion as a separate unit superficially resembles the "rhizomycelium" of the other polycentric chytrids with the nuclei confined to the replicated swellings and the presence of anucleate isthmuses and rhizoids. However, these two species differ from the others in that no reproductive organs are produced from the swellings of the vegetative portion. A possible exception to this is that Karling's (1941) original description of *N. profusa* reports the formation of resting bodies formed directly from the intramatrical swellings. The present strain of *N. profusa* is sterile in this respect. Since reproductive bodies may be formed intramatrically on short filaments which do not extend out of the cells of decaying grass leaves, it might be that the resting bodies described by Karling, as well as those by Whiffen (1943) for *N. delica*, were not transformed true vegetative swellings.

The sterile swellings confined to the vegetative portion of *N. ramosa* and *N. profusa* appear to be similar to the intramatrical prosporangial (the term is used here in the sense of Whiffen, 1944)

swellings such as are found in *Nephrochytrium*, especially those forms which are lobed. Karling (1938) suggested that this monocentric genus might be a stage in the evolution of the polycentric mode of development. If this conjecture were acceptable the flexuous filaments on which the reproductive organs are borne would be elongated and branched homologues of the isthmuses between the intramatrixal prosperangia and the extramatrixal zoosporangia. Such a hypothesis is substantiated to some degree by the occasional formation of a single sporangium close to the substratum on a short filament which extends only from the intramatrixal swelling in the adjacent cell of the decaying leaf. The swellings in the extramatrixal filaments might be the result of progressive sterilization accompanying the process of elongation.

The term "rhizomycelium" does not seem to characterize adequately the reproductive portions of these two strains. The filaments are specialized outgrowths of the vegetative portion and are not intimately associated with rhizoids in structure or function. Unlike the typical rhizoids of the chytrids, they do not become completely devoid of cytoplasm, nuclei, and food materials during the production of a single zoosporangium; but retain enough to produce secondary, tertiary, and chains of zoosporangia in basipetal succession (especially in *N. profusa* which is lacking numerous swellings in these filaments). Unlike the case in the "rhizomycelium" of *Cladochytrium replicatum*, the nuclei are not confined in the swellings of these sporangium-bearing filaments.

The vegetative portion persists throughout the life of the thallus as an absorptive and storage unit, so that a complete change from a trophocentric to a genocentric thallus does not occur. In this manner the thallus of *Nowakowskiella* resembles the true mycelium of the higher fungi, and continues to digest the substratum and produce asexual spores until the available food supply has been diminished or the concentration of metabolic waste in the medium inhibits growth.

Likewise, similar to the higher fungi, when the culture has aged *N. ramosa* produces fewer asexual spores, and initiates resting cell formation. So far as it has been possible to determine, the production of resting cells in this species is not preceded by nuclear fusion. Karling (1945c) refers to the fusion of filaments before the forma-

tion of the pseudoparenchyma as "vegetative anastomosing." Shanor (1942a) presented some evidence that a fusion nucleus may be present in the resting cell of *N. hemisphaerospora*. The same might be true of *N. ramosa*, for the cytoplasmogamy in *N. hemisphaerospora* bears some resemblance to the formation of the first cell of the pseudoparenchyma in *N. ramosa*. The formation of "parthenogenetic" resting spores has been reported for the genus *Siphonaria*, a genus which supposedly demonstrates sexuality among the chytrids (Karling, 1945c).

In general, the two species of *Nowakowskiella* studied do not differ greatly in behavior from that described for other chytrids in regard to the nuclei and cytoplasm during the development of the thallus and the formation of reproductive organs. Such differences as do occur may be primarily due to the specialization of the portions of the thallus and the extensive extramatrix development.

The most pronounced characteristic by which *N. ramosa* and *N. profusa* differ from the other polycentric chytrids is the distinct differentiation of the thallus into vegetative and reproductive portions, both of which persist in a functional state.

As to the relationships of *Nowakowskiella*, little can be ventured until other members of the operculate series of the Chytridiales are investigated more fully. In general habit, except for the intramatrix swellings and in the possible fusion of filaments preceding the formation of resting cells, this genus might suggest an incompletely known phylogenetic series between the Chytridiales and the Zygomycetes. Until much more is known about the trophic mycelium as compared with the reproductive mycelium of the latter and until possible transition forms have been investigated, this hypothesis can be considered merely as an interesting speculation.

SUMMARY

1. The growth of *Nowakowskiella ramosa* and *N. profusa* is abundant on solid substrata composed primarily of cellulose if the surrounding liquid contains organic decomposition products, such as found in river water, pond water, etc.
2. The natural optimum temperature for the development of *N. ramosa* is between 16° and 18° C. and that of *N. profusa*, 24° and 28° C. Both species may be adapted to cultivation at other temperatures.

3. The cell walls of both species show a predominance of chitin which is mixed with cellulose in the same layer. The opercula are pectinaceous.
4. The behavior of the protoplasm during development of the thallus and formation of the reproductive organs does not differ greatly from that in other chytrids.
5. The flagellum appears to be formed within the sporangium and is connected with the nucleus of the zoospore by a rhizoplast near one of the points of a tilted nuclear cap.
6. The center of growth in the developing thallus is early transferred from the spore case to a swelling in the germination tube.
7. The mature thallus is divided into two distinct portions which function concurrently, the vegetative portion and the reproductive portion.
8. The complete thallus is not adequately characterized by the term "rhizomycelium" which has been applied to the thalli of the polycentric chytrids.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Distinguished Professor of Botany Ernest A. Bessey of Michigan State College for his helpful guidance and suggestions in the completion of the investigations and his careful correction of the manuscript. Thanks are also given to Professor Richard de Zeeuw for his advice in methods of staining and mounting specimens.

EXPLANATION OF FIGURES

FIG. 1. *Nowakowskiella ramosa*. *a*. Living zoospores. *b*. Stained zoospores. *c*. Typical spores after having become quiescent. *d*. Miniature non-flagellate spores. *e*. Germination of spore. *f*. Vegetative portion of thallus. *g*. Flexuous filaments. *h*. Uni- and binucleate zoosporangium initials. *i*. Mitotic division in a trinucleate zoosporangium initial. *j*. Sporangium initial with granular strands and beginning of cross-septum. *k*. Sporangium, with mature wall and operculum, in which vacuolar cleavage has begun. *l*. Sporangium showing complete cleavage. *m*. Sporangium showing aggregation of granules about nuclei. *n*. Mature sporangium showing compact nuclear caps. *o*. Release of zoospores, showing flagella in zoosporangium and one spore held by a looped flagellum. *p*. Early stages in plasmogamy, preceding formation of the pseudoparenchyma. *q*. Pseudoparenchyma with resting cell initials. *r*. Mature resting cells.

FIG. 2. *Nowakowskiella profusa*. a. Living zoospores. b. Stained zoospores. c. Germination of spore. d. Vegetative portion of thallus. e. Flexuous filament showing pseudosepta at constrictions. f. Typical flexuous filament. g. Stages in zoosporogenesis drawn from living material. h. Stages in zoosporogenesis drawn from stained material. i. Reduced sketches of shapes of proliferating and elongate zoosporangia. j. Late stage in zoospore escape, showing looped flagella and hyaline pseudopodia.

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MYRIOGONIUM, A NEW GENUS AMONG SIMPLIFIED ASCOMYCETES¹

ROY F. CAIN²

(WITH 56 FIGURES)

Because of their supposed significance in the evolution of the Ascomycetes the non-ascocarpous species of this group are of special interest. While examining a specimen of *Odontia sudans* (Alb. & Schw., ex Fr.) Bres. collected July 29, 1933 by the late Professor V. Litschauer of Innsbruck, Austria, the writer noticed a peculiar associated Ascomycete. In habit this fungus somewhat resembles *Helicogonium Jacksonii* which was described by W. L. White (4) from collections made on *Corticium microsporum* (Karst.) Bourd. & Galz. in Ontario. More detailed study showed that it is quite different and apparently undescribed.

Myriogonium gen. nov.

Ascocarpio nullo; mycelio vegetativo parco, hyphis ramosis, septatis; cellulis terminalibus (gametangiis) binis conjungentibus; cellula ab fusione creta ascos multos in cymbiformi fasciculo gignente; ascis octosporis, elongatis, sessilibus, basi septo cum hamo separatis, apice incrassato, obtuso, in maturitate aperto; ascosporis hyalinis, unicellularibus, elongato-ovatis.

Ascocarp lacking; vegetative mycelium scanty, the hyphae branched, septate, the terminal cells acting as gametangia but not distinctly differentiated; fusion cell forming several asci in a unilaterally cymose cluster; asci eight-spored, elongate, sessile, separated from fusion cell by a septum with hook, the apex thickened, obtuse, liberating spores through an opening at apex; ascospores hyaline, one-celled, elongate-ovate.

¹ Contribution from the Department of Botany, University of Toronto.

This study was carried out with the assistance of a grant in aid of research provided by the University of Toronto.

² The author is greatly indebted to Professor H. S. Jackson for suggestions and criticism of the manuscript and to Miss D. F. Vick for copying and inking the drawings.

Myriogonium Odontiae sp. nov.

Ascocarpio nullo; mycelio vegetativo parco, plerumque in hymenio hospitis sed in subhymenium extendente; hyphis hyalinis, flexuosis, profunde ramosis, propinque septatis, tenui tunicatis, ad septum constrictis, cellulis $5.0-10 \times 1.0-2.5 \mu$; cellulis terminalibus subincrassatis, binis conjungentibus, ambabus rarer in duabus ramis ejusdem hyphae cretis sed saepissime in duabus ramis dissimilium hypharum gestis; cellula ab fusione creta sine septo tenente et apice tumorem gignente; asco ex cellula subultima typici hami gerente; cellula ultima cum cellula ad basem conjungente; hac cellula hamos et ascos continenter gerente; ascis initio ovatis, tum elongatis, maturitate late clavatis vel subcylindraceis, sessilibus, octosporis, $18-25 \times 5-7 \mu$, apice obtuso-rotundato, foramine lato ad ascosporarum emissionem apertante, incolorabili iodi ope; sine paraphysibus; ascosporis hyalinis, elongato-obovatis vel prope ellipsoideis, sursum latius rotundatis, $4.5-6.0 \times 1.2-1.7 \mu$, unicellularibus, oblique distichis.

Ascocarp lacking; vegetative mycelium scanty, mostly in hymenium of its host but extending into subhymenium; hyphae hyaline, flexuous, profusely branched, closely septate, thin-walled, constricted at the septa, with cells measuring $5.0-10 \times 1.0-2.5 \mu$, terminal cells becoming somewhat swollen and fusing in pairs, usually from separate but occasionally from the same branching system; two fusing cells remain continuous and grow out by means of a swelling at apex of one of fusing cells; an ascus is formed from penultimate cell of a typical crozier; the ultimate cell fuses with basal cell which remains continuous with fusion cell; this continuing to produce croziers by proliferation in the region of the ultimate cell so that a small group of asci are formed each from a penultimate cell in a unilaterally cymose cluster; asci at first ovate, elongating, at maturity broadly clavate to subcylindric, sessile, eight-spored, $18-25 \times 5-7 \mu$, the apex obtuse, rounded, opening to discharge ascospores through a wide pore at apex, not colored by iodine, paraphyses lacking; ascospores hyaline, elongate-obovate to nearly ellipsoid, more broadly rounded at upper end, $4.5-6.0 \times 1.2-1.7 \mu$, one-celled, obliquely biserial or somewhat irregularly arranged.

On *Odontia sudans* (Alb. & Schw., ex Fr.) Bres. on decaying wood of coniferous tree. Hochmoos bei Platzl in der Leutasch, Tirol. July 29, 1933. V. Litschauer (77). Type (in University of Toronto Cryptogamic Herbarium).

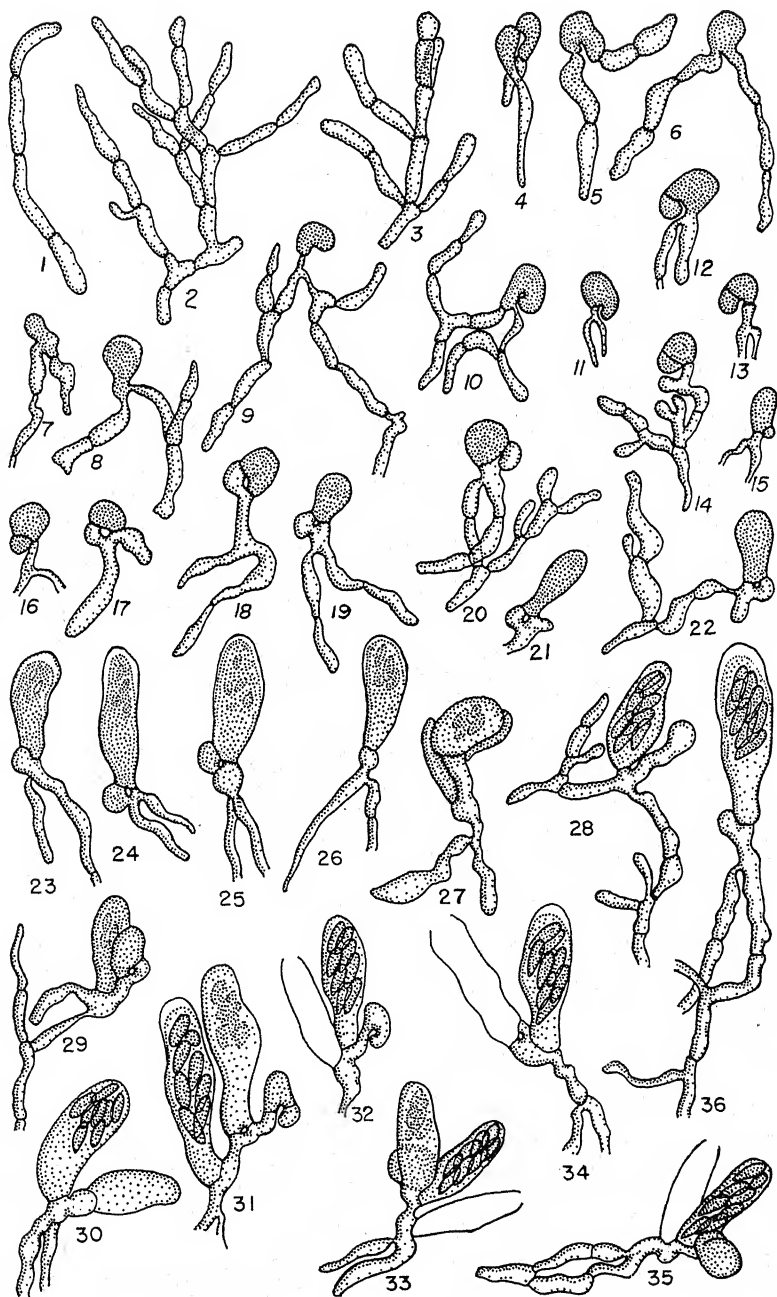
From the location of the asci the fungus appears to be parasitic on the *Odontia*, and covers a little more than half of the area included in the collection studied. There is no change in the appearance of the *Odontia* due to the presence of the Ascomycete

that is visible to the naked eye or evident under a high power wide field binocular microscope. Asci have not been found in the extreme marginal area but in some places occur a few millimeters away. They become more abundant in the older areas and in some places the hymenium of the Basidiomycete is entirely replaced by the parasite. In the newly infected areas asci are found scattered in the same field as basidia bearing normal basidiospores. Some areas of mature hymenium several centimeters in diameter are entirely free from asci.

There is nothing in the appearance of the specimen to suggest that the Ascomycete might have developed during or subsequently to drying of the fresh material. Only the one packet of the collection has been available for examination. It seems probable that the portion of the same collection in Litschauer's herbarium would also bear the Ascomycete.

The ascomycetous hyphae (FIGS. 1-3, 54, 56) are entirely different from the clamped hyphae of the host (FIG. 56) in being narrower with short cells constricted at the septa. These could not be traced beyond the subhymenium of the host and are found most abundantly in the hymenium (FIG. 56). No connection between the two sets of hyphae could be found. The young branching hyphae are most abundant in the newly infected areas. In the older portions these are largely replaced by the clusters of asci with the fusion cell and two chains consisting of three or four vegetative cells each. The hyphae in the hymenium appear to arise at right angles from more elongate hyphae with less frequent branching located in the subhymenium (FIG. 54).

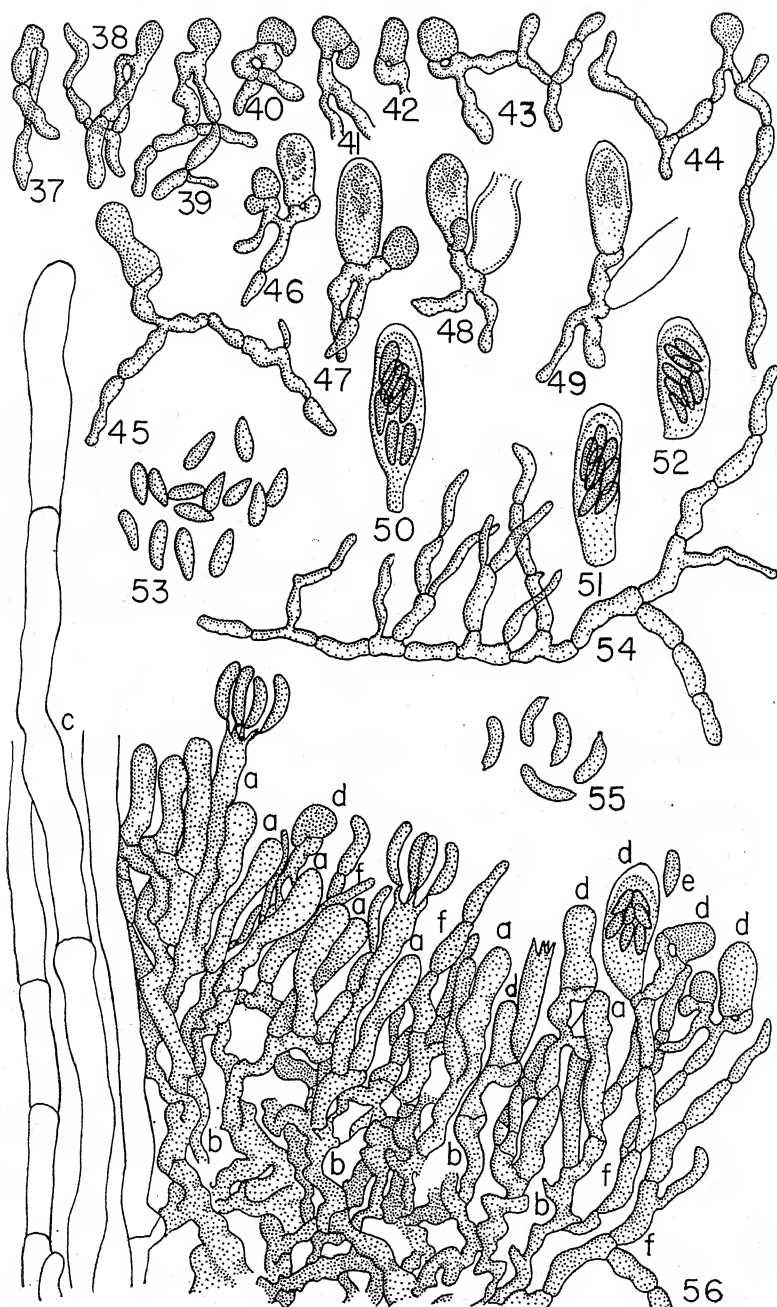
The fungus appears to be distinctly different from and perhaps not immediately related to *Helicogonium Jacksonii* in lacking anything that can be interpreted as ascogonia and antheridia. Fusion cells are produced by the growing together of two terminal cells of the branches (FIGS. 4-7, 37-39). These are not separated from the two fusing gametangia by septa so that they appear with a two-pronged base (FIGS. 5-6) and are produced in great abundance. Apparently any of the terminal cells of the branched hyphae may function as gametangia. Fusion usually takes place between cells of different branching systems (FIGS. 6, 9, 10, 28, 39, 44) but a few have been found in which the two gametangia could be traced



FIGS. 1-36. *Myriogonium Odontiae*.

back to the same branch one or two cells below (FIG. 35). Gametangia about to fuse (FIG. 4) or which have just united (FIGS. 5, 37) are difficult to find. In the few cases that were seen they were distinctly swollen, the one sometimes curved to meet the other. All stages of development subsequent to fusion could be found in abundance. These show no evidence of any swelling or of any appreciable differentiation between the two fusing cells (FIG. 5). A distinct constriction is usually seen where the one has fused with the other. The two fusing cells remain with a continuous cavity. Near the point of fusion a swelling takes place in the wall of one of the original gametangia which is typically separated from the other by a narrow constriction, but no septum is developed (FIGS. 5, 37). This swelling becomes ovate or subglobose (FIGS. 6-8, 38-39) and then elongates and curves into a somewhat reniform structure (FIGS. 10-11, 40), and develops into a typical crozier. A septum cuts off a basal cell which remains continuous with the forked fusion cell (FIGS. 11, 40). A second septum cuts off an ultimate cell (FIGS. 12-16, 41) which fuses with the basal cell (FIGS. 17-18, 42). The penultimate cell enlarges on the distal side (FIGS. 19-22, 42-45) and always develops into an ascus (FIGS. 23-26). This is followed by an outgrowth in the region of the ultimate cell (FIG. 28) which develops into a crozier (FIG. 29) which produces a second ascus (FIG. 34) in a manner similar to the first. This process of crozier formation is continued so that there is produced a unilaterally cymose cluster of asci which mature in succession (FIGS. 31-32). Each cluster usually has a crozier, a partially developed ascus, a mature ascus and one or two empty ones. Swellings in the basal cell below often show that several other asci have matured and disintegrated (FIG. 34). There are no paraphyses and apparently all of the terminal cells of the vertical branches are used in the production of gametangia as few or none are left in the older portions.

Such a system of ascus formation is adapted to the gradual and continuous development of asci and in this case probably only limited by the exhaustion of the food supply of the host. By this method the penultimate cell of each crozier always develops into an ascus.



FIGS. 37-56. *Myriogonium Odontiae*.

This method of development is similar to that described by Rogers (3) for basidial proliferation in *Sebacina prolifera* Rogers. Here the cell which corresponds to the penultimate cell of a crozier always develops into a basidium while renewed growth takes place from the stalk in the region of the beak. In this manner an indefinite series of basidia maturing in succession is formed and probably limited only by failure of food supply or unfavorable weather conditions. This is in marked contrast to the method of ascus formation by croziers in a species such as *Aspergillus Fischeri* Wehmer. In this species according to Olive (2) there is a proliferation of ascogenous cells by means of croziers which develop from both the penultimate cells and the crozier tip. The distinction here is that the penultimate cells of the croziers develop into new croziers instead of asci. Not until the ultimate growth of the mass of ascogenous hyphae has been reached do the penultimate cells cease forming croziers and develop into asci. Such a system results in the more or less simultaneous development of all of the asci within the ascocarp.

According to Rogers (3) in *Pyronema confluens* (Pers.) Tul. the ascogenous hyphae in the early stages of their development proliferate by the formation of a new crozier from the penultimate cell of the previous crozier. Only in later development is this process terminated by the formation of an ascus instead of a crozier from the penultimate cell. At this stage new croziers are produced from the region of the basal or ultimate cells.

Many who adhere to the doctrine of the evolution of the Ascomycetes from some simple representative such as *Dipodascus* to the more complex types with elaborate ascocarps, may consider *Myriogonium Odontiae* as a representative in the early series of such an evolution. No such significance is attributed by the author to this fungus. On the contrary, it is considered as a highly specialized member in a terminal series of morphologically reduced forms, the reduction in morphology being associated with its highly specialized habitat. While its actual relationships are uncertain its ancestral forms are to be sought among the more elaborately organized fungi, possibly among the inoperculate Discomycetes. It seems quite possible that *Helicogonium* may also represent a

simplified form derived from some higher Ascomycete line rather than belonging among the Hemiascomycetes.

Myriogonium differs from *Helicogonium* in several important respects, especially in having one-celled ascospores which do not bud in the ascus, in lacking ascogonia and antheridia and in possessing croziers. In *Myriogonium* fusion takes place between hyphal tips, or only very slightly specialized branches of the hyphae. This may represent the result of a reduction from some more elaborate type of gametangia or might be interpreted as hyphal anastomoses where fructification results at each point of fusion. The fact that *Helicogonium* lacks croziers cannot be taken to indicate a complete lack of relationship. Species in the same genus may or may not have croziers. Emmons (1) has shown that in *Thielavia terricola* (Gilman and Abbott) Emmons the asci arise from the penultimate cell of a crozier while in *Thielavia Sepedonium* Emmons they arise as simple side branches from the ascogenous hyphae without crozier formation. A similar situation is reported by White (5) for *Helotium*, a genus of inoperculate Discomycetes. In *Helotium albidum* (Rob., ex Desm.) Pat. and *H. Dearnessii* (Ell. & Ev.) White the asci do not originate from croziers. In such species as *H. citrinulum* Karsten, *H. midlandensis* White, *H. erraticum* White, *H. scutula* (Pers., ex Fr.) Karst. var. *fucatum* (Phill.) Rehm and *H. gemmarum* Boud. the asci develop from croziers.

Within the Basidiomycetes there are many related groups of species some of which have clamped hyphae and some lack clamps. It is generally considered, by students of this group, that the species lacking clamps have been evolved from the clamp-bearing species. In a similar manner the ascomycetous species lacking croziers have probably developed from forms in which they are present.

It is perhaps worth while to point out that the "fungus" here described is not a single individual as it is found on its host nor a single generation. The vegetative mycelium is the haploid generation and consists of one individual plant (if from a single spore) or several individual plants growing together (if from two or more spores). Each cluster of asci together with the fusion cell from which it arises represents an individual plant of the dikaryotic

diploid generation. Each ascospore represents the beginning of a new haploid individual.

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5. —. Studies in the genus *Helotium*. IV. Some miscellaneous species. Farlowia 1: 599-617. 1944.

EXPLANATION OF FIGURES

All figures were drawn with the aid of a camera lucida from preparations of crushed material mounted in potassium hydroxide-phloxine. Figs. 1-36 drawn at a magnification of 1600 and reduced in reproduction to 1070. Figs. 37-56 drawn at a magnification of 2100 and reduced to 1120.

Figs. 1-3. Branched hyphae. Fig. 4. Two separate branches just before fusion of the terminal cells or gametangia. Fig. 5. Fusion cell just after fertilization. The upper part of the fusion cell has produced a small swelling, the early stage in the formation of the ascus hook. Figs. 6-9. Early stages in the formation of the outgrowth from the fusion cell. Figs. 10-11. The outgrowth has elongated and curved into a kidney-shaped structure, the ascus hook. Fig. 12. The hook has cut off a terminal cell. Figs. 13-16. Two septa have formed to divide the terminal or ultimate cell and the penultimate cell from the fusion cell. Figs. 17-18. The terminal cell has fused with the basal or fusion cell. Figs. 19-26. Stages in the development of the first ascus from the penultimate cell. Fig. 27. Third ascus has begun to form. Fig. 28. The ultimate cell of the first hook, after uniting with fusion cell, has grown out prior to the formation of the second hook. Fig. 29. The second ascus has begun to enlarge from the penultimate cell of the second ascus. Fig. 30. The first ascus has mature spores and the second one partly developed. Fig. 31. The ultimate cell of the third hook is about to fuse with the basal cell which remains continuous with the basal cells of the second and first hooks as well as the fusion cell. Figs. 32-35. The first ascus has discharged its ascospores through a pore in the apex. Fig. 36. A mature ascus with the fusion cell formed from two gametangia, the terminal cells of two branches borne on separate hyphal systems. In Fig. 35 the gametangia were the terminal cells of two-celled branches originating on the same hyphal cell.

Fig. 37. Two separate branches with two cells each; the terminal ones (gametangia) have fused. *Figs. 38-39, 44.* Development of the swelling from the fusion cell. *Fig. 40.* The basal cell (which is continuous with the fusion cell) is cut off by a septum. *Figs. 41-43.* The penultimate cell is separated by septa from the basal and terminal cells. *Fig. 45.* Enlargement of the penultimate cell of the first hook to form the first ascus. *Figs. 46-47.* The second ascus beginning to develop and spores being delimited in the first. *Figs. 48, 49.* Empty ascus with others in various stages of development. *Figs. 50-52.* Asci with mature spores and thick-walled apex. *Fig. 53.* Ascospores after discharge from asci. *Fig. 54.* Vertical branching hyphae with terminal gametangia growing from a vertical vegetative hypha. *Fig. 55.* Basidiospores of *Odontia sudans*. *Fig. 56.* Section of *Odontia sudans* taken near the apex of a tooth of fruiting body. *a.* young basidia; *b.* hyphae bearing basidia; *c.* cystidia of host forming axis of tooth and projecting at apex; *d.* asci in various stages of development; *e.* ascospores; *f.* branched hyphae of *Myriogonium*.

MARTENSELLA CORTICII THAXTER AND ITS DISTRIBUTION¹

H. S. JACKSON AND E. R. DEARDEN

(WITH 11 FIGURES)

Incidental to the study and identification of several thousand Canadian collections of corticioid Basidiomycetes during the past fifteen years in the mycological laboratory of the University of Toronto, a number of interesting associated fungi have been encountered, some or all of them parasitic on the Basidiomycetes.

One of the first of these to attract attention was found growing on the surface of a fructification of *Corticium bombycinum* (Somm.) Karst. and was described and illustrated by the late Dr. David H. Linder (4) as *Spondylocladiella botrytioides*, a new genus and species of dematiaceous Hyphomycete. A second form, found several times growing on *Corticium microsporum* (Karst.) Bourd. & Galz., proved to be a simple Ascomycete producing individual clavate asci scattered among the basidia in the hymenium of the *Corticium* in such a manner that, when first observed in an immature condition, they were mistaken for the cystidia of a *Peniophora*. The collections of this Ascomycete were finally sent to Dr. W. L. White (8) who described the parasite as *Helicogonium Jacksonii*, a new genus and species which he assigned, perhaps incorrectly, to the Hemiascomycetes. *Platyglaea Peniophorae* Bourd. & Galz., found associated with fructifications of *Peniophora*, was first reported from North America by Dr. G. W. Martin (6), based on Canadian collections sent him from this laboratory. In the same paper Martin also described as new species *Tremella mycophaga* on *Aleurodiscus amorphus* (Pers.) Rabenh. ex Cooke, and *Tremella simplex* Jackson & Martin on an as yet undescribed *Aleurodiscus* on *Thuja occidentalis*, both from Ontario. Recently the

¹ Contribution from the Department of Botany, University of Toronto.

This study was carried out with the assistance of a grant in aid of research provided by the University of Toronto.

senior author (3) has described, as a new genus and species, *Trichomonascus mycophagus*, a remarkable Ascomycete of uncertain relationship found growing from the hymenium of *Corticium confluens* Fr. Still more recently Dr. R. F. Cain (2) has published, also as a new genus and species, *Myriogonium Odontiae*, another unusual Ascomycete found associated with the hymenium of *Odontia sudans* (Alb. & Schw., ex Fr.) Bres. This was discovered on a collection of the host in the herbarium of the University of Toronto, made in Austria by the late Professor V. Litschauer. The senior author has in manuscript an undescribed species of *Helicobasidium* found growing in relation to a species of *Peniophora*. Several other collections of auriculariaceous fungi growing in association with resupinate Thelephoraceae await further study.

MARTENSELLA CORTICII Thaxter

✓ In addition to this rather remarkable series of associated fungi, another form was observed first in the fall of 1945 by the junior author, growing on the hymenium of *Corticium radiosum* Fr. This proved to be a Phycomycete, *Martensella Corticii* Thaxter, described as follows in a recent paper by Linder (5, p. 59) and assigned to a new family, the Kickxellaceae, of the Mucorales.

"Colonies effuse, forming a minutely and sparsely hirsute layer over the substratum, pale yellowish or 'Cream Color.' Conidiophores simple, hyaline, 1-3-septate, up to $200\ \mu$ long, $5.5\text{--}6.5\ \mu$ in diameter and bearing one or two, rarely three, sporocladia. Sporocladia stipitate, the stalk cell $9\text{--}12.5 \times 4.5\ \mu$, the sporocladia (2)-3-4-(5)-septate, $25\text{--}36\ \mu$ long, inflated in the fertile portion where they are $7\text{--}10\ \mu$ in diameter, somewhat tapering to the bluntly rounded base and the sharply upturned sterile apex. Phialides 6-8 on the upper surface of the sporocladium, $5\text{--}6 \times 3.5\ \mu$. Conidia elongate-ellipsoid to subcylindrical, slightly tapering towards the base, rounded towards the apex, $10.5\text{--}13 \times 3.5\text{--}4.5\ \mu$.

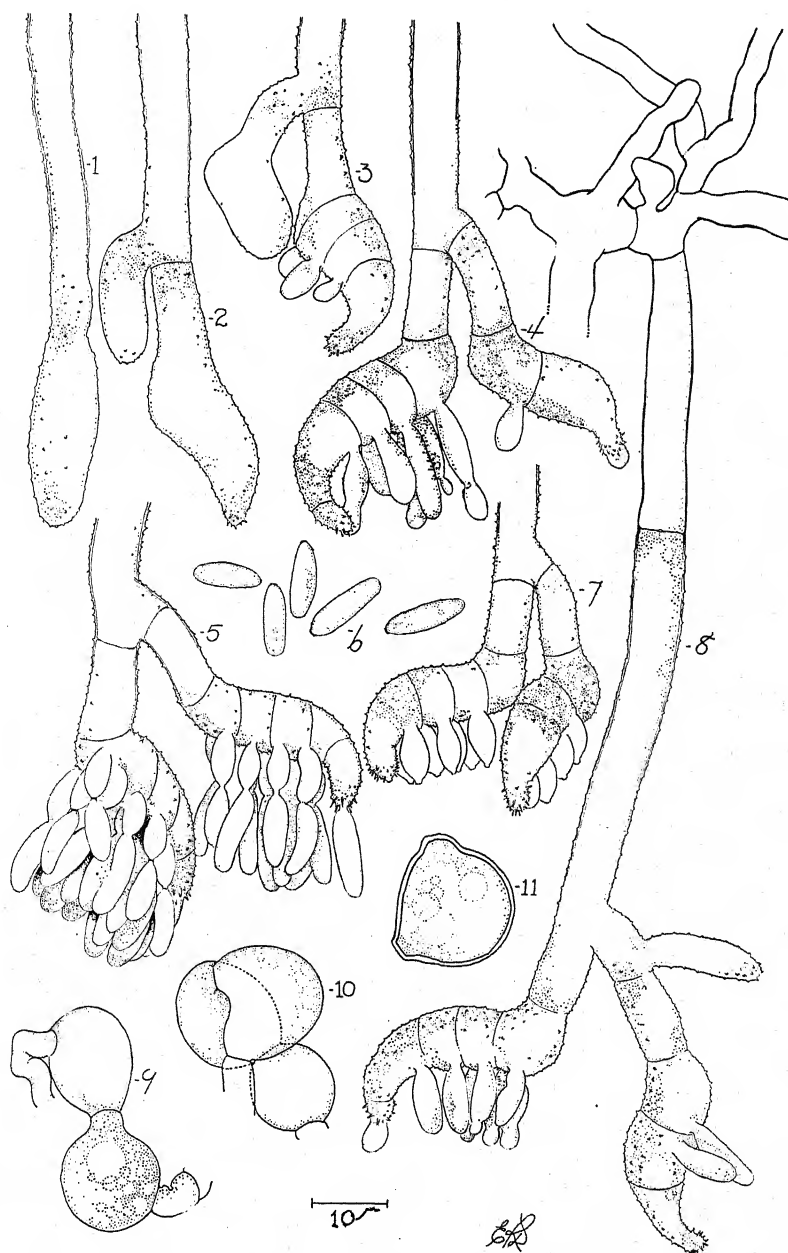
Specimens examined: **New Brunswick**, Campobello, on *Corticium* sp., W. G. Farlow, **type**; **New York**, Enfield Gorge near Ithaca, on *Corticium alutaceum*, Oct. 18, 1902, G. F. Atkinson."

The above description was checked with the type and other collections. It is not entirely satisfactory, and requires several amendments. Contrary to the description, the apex of the sporo-

cladium is not always sterile but may function as a phialide bearing a single conidium (FIG. 5). As a minor omission, it should be noted that the walls of the aerial portion of this fungus are delicately asperulate, and the apices of the sporocladia echinulate. The conidia and vegetative portions of the fungus are smooth walled.

As a major omission attention should be drawn to the presence of structures, foreign to the host, consistently associated with the conidial stage of the Phycomycete. These are found imbedded within the Basidiomycete and at maturity are subglobose, 10–20 μ in diam., smooth and hyaline. The majority of these in the type collection have thin walls, but many in other collections have the walls thickened 1.5–2 μ . Produced singly, or more commonly in chains of 2–5 as intercalary swellings on the vegetative hyphae, they are best interpreted as chlamydospores (FIGS. 9–11). While the organic connection between the aerial conidial phase and the chlamydosporic phase of the mycelium has not been traced, the consistent association in over thirty collections of *Martensella Corticii* from widely distributed localities is submitted as convincing evidence of this connection. It should be emphasized that these structures are of an asexual nature. There is no evidence of a union with any other hyphal branch. That they are borne in chains is evidence of their vegetative origin.

In this connection, however, attention should be drawn to the zygosporos described and illustrated by Linder for a related species, *Coemansia aciculifera*. This species had been obtained by Thaxter, according to the evidence available, from germinating zygosporos found in Sphagnum. The structures which are referred to above as chlamydospores in *M. Corticii* are certainly not to be interpreted as zygosporos or as azygosporos. Possibly these correspond to what Linder, in his discussion of *C. aciculifera*, refers to as gangliform swellings on the mycelium. Unfortunately he furnished no figure of these structures and does not describe them in any detail but compares them with structures figured by Van Tieghem for *Mortierella tuberosa* V. Tieg. Linder recognizes clearly the uncertainty of the relationship of the fungi of this group to the Zygomycetes when based on the somewhat uncertain evi-



FIGS. 1-11. *Martensella Corticii*; 1-5, stages in the development of conidiophores with primary and secondary sporocladia; 6, conidia; 7, discharged conidiophore; 8, conidiophore with a tertiary sporocladium developing; 9-10, young chlamydospores in chains; 11, a mature chlamydospore.

dence of zygospores and more reasonably places the Kickxellaceae there on comparative morphological grounds.

All attempts to obtain cultures from the conidia have so far resulted in failure. Special media have not yet been used and no attempt to grow the fungus from chlamydospores has been made.

DISTRIBUTION

It appears from the evidence available that *Martensella Corticii* occurs only on *Corticium radiosum* Fr. and is probably parasitic, perhaps obligately so on that host. The previously unidentified host of the type proves to be that species. *Corticium alutaceum* (Schr.) Bres., cited by Linder as the host for the single paratype, is synonymous with *C. radiosum* according to Burt (1, p. 263).

Following the first recognition of the parasite in Ontario a number of other field collections have been made but most of the records from that province and elsewhere have been obtained by a careful examination of the considerable number of specimens of *C. radiosum* which were already filed in the University of Toronto herbarium. To determine the distribution of *M. Corticii* more accurately all the specimens of *C. radiosum* and of *C. lacteum* Fr. in the Burt and general herbaria at the Farlow Herbarium were examined through the courtesy of Dr. Rolf Singer. *C. lacteum* was included for examination because Burt had confused it with *C. radiosum* (7, p. 294). Similarly the collections in the Lloyd Herbarium and in the Herbarium of the Bureau of Plant Industry were loaned by Dr. J. A. Stevenson and the specimens in the Overholts Herbarium and the portion of the Burt collection at the Missouri Botanical Garden were loaned by Drs. F. D. Kern and C. W. Dodge respectively. Five additional records were obtained from these herbaria.

During this survey all collections were examined carefully with a wide field binocular microscope for the hirsute, orbicular, frequently coalescing colonies of the parasite. The characteristic appearance of these colonies overgrowing the perfectly smooth hymenium of the Basidiomycete makes such an examination relatively simple. Subsequently determinations of *M. Corticii* were confirmed by examination with a compound microscope.

The distribution of *M. Corticii* in North America as revealed by these studies is widespread. Some thirty-one collections are deposited in the University of Toronto Herbarium. These records are from four Canadian provinces and seven American states. None of the limited number of European collections of *C. radiosum* available was parasitized. Although at present known only from North America, *M. Corticii* may perhaps be expected wherever its host, a cosmopolitan species, is found.

COLLECTIONS EXAMINED²

Ontario: S. of Aurora, Oct. 5, 1934. R. F. Cain, 21570 from 5433; Sept. 29, 1946. H.S.J., 21565 from 21293; Benwell swamp, Gobles, Oxford Co., Sept. 24, 1939. R. F. Cain, 21572 from 15074; S. of Hatchley, Brant Co., Oct. 27, 1934. R. F. Cain, 21573 from 5439; Sept. 25, 1937. R. F. Cain, 20124 from 12571; W. of Maple, Oct. 1946. H.S.J., 21566; Nashville, York Co., Oct. 13, 1945. R. F. Cain, 20121 from 20204; Ottawa, 1891. J. Macoun, from MoBGH 44640; Oxtongue Lake, 5 mi. W. of Algonquin Park, Oct. 14, 1940. H.S.J., 21862 from 16423; N. of Richmond Hill, Sept. 28, 1934. H.S.J., 21861 from 5497; Oct. 3, 1935. R. F. Cain, 20123 from 8228; Sept. 29, 1937. H.S.J., 20125 from 12108; Oct. 14, 1937, H.S.J., 21571 from 12565; Silver Lake, Frontenac Co., Sept. 1, 1941. R. F. Cain, 20126 from 17805; Timagami, Aug. 1907. C. G. Lloyd, 20127 from LH 16005; Paradis' Bay, L. Timagami, Sept. 3, 1936. R. Biggs, 20122 from 10116.

Quebec: Lower St. Lawrence Valley, Oct. 13, 1905. J. Macoun, from MH 87, in Herb. Burt FH; Indian Lake, near Wilson's Corners, Oct. 12, 1935. I. Mounce from F 6713, ex Myc. Herb., Ottawa.

New Brunswick: Campobello, W. G. Farlow, Type, FH.

British Columbia: Clearwater, Oct. 4, 1944. D. C. Buckland, from Myc. Herb. Ottawa 16182, ex V-934 Dom. Lab. Forest Path. Victoria. B. C.

Connecticut: Woodbridge, Oct. 2, 1933. J. R. Hansbrough, from USDA, FP 81254; Branford, Nov. 10, 1933. J. R. Hansbrough, from USDA, FP 84036; Cockaponset State Forest, Chester, Nov. 21, 1937. H. G. Eno, from USDA, FP 82476; Nov. 2, 1937. H. G. Eno, from USDA, FP 82479.

New York: Enfield Gorge, near Ithaca, Oct. 18, 1902. G. F. Atkinson, from CU 14101, Paratype, FH; Warrensburg, Oct. 4, 1931. H. D. House, TRT.

² Unless otherwise stated, the collection numbers given below are those of the Cryptogamic Herbarium of the University of Toronto (TRT). Where two numbers are given, separated by the word "from," the first is the segregated specimen of *Martensella* and the second that of the host collection.

Pennsylvania: State College, Mar. 11, 1918. L. O. Overholts, from OH 4691, Penn. State Coll. Herb.; Emlenton, Butler Co., Oct. 6, 1934. L. O. Overholts and W. A. Campbell, from OH 19291, Penn. State Coll. Herb.

Michigan: New Richmond, Nov. 22, 1913. C. H. Kauffman, from MoBGH 22870.

Arizona: Santa Catalina, Coronado Nat. Forest, Oct. 15, 1911. G. G. Hedgcock and W. H. Long, from USDA, FP 9787.

Utah: S.E. of Ephraim, Sept. 19, 1902. G. G. Hedgcock, from USDA, FP 3968.

Wyoming: So. Brush Creek Camp Ground, Medicine Bow Nat. Forest, July 25, 1942. S. M. Pady, TRT.

DISCUSSION

The three genera of the Kickxellaceae fall naturally into two groups; the genus *Kickxella* is characterized by a whorled arrangement of the sporocladia about the apex of the conidiophore whereas the genera *Coemansia* and *Martensella* are characterized by a diffuse arrangement of the sporocladia along the simple or branched conidiophores. Of the two latter genera, according to Linder, the generic separation is based on the orientation of the phialides; along the upper surface of the sporocladia in *Martensella* and on the lower surface in *Coemansia*. In placing *M. Corticii* in *Martensella* rather than *Coemansia* the orientation of the host to its substrate was apparently not considered. Fructifications of resupinate Basidiomycetes are commonly found in nature on the lower surface of their substrata. If we are to evaluate this factor, it is evident that the aerial portions of a parasite colonizing a *Corticium* must project downwards. It follows then that the conidia and phialides of *M. Corticii* are pendant from the downward projecting conidiophores and are actually arranged along the lower surface of the sporocladia. It may be argued that this arrangement is simply an adaptation for purposes of dissemination of the conidia, and may be interpreted as of no more significance than a geotropic response.

The method of proliferation of sporocladia in *M. Corticii* (FIGS. 1-5, 8) offers further evidence of the close relationship to species placed in *Coemansia*. Conidiophores of *M. Corticii* bear normally two or rarely three sporocladia. The primary sporocladium develops from a terminal enlargement of the conidiophore (FIG. 1).

This develops asymmetrically and becomes separated by a septum from the supporting conidiophore which then proliferates laterally immediately behind the septum (FIG. 2). A secondary sporocladium develops at the apex of the lateral outgrowth and is also separated by a septum from the supporting conidiophore (FIGS. 3-4). A tertiary sporocladium is occasionally proliferated from immediately behind the secondary one. Further development of the sporocladia follows a common pattern. Each becomes 2-4-septate and produces 2-4 finger-like phialides from all but the first and last cells. These projecting phialides in turn produced conidia as apical swellings. The first cell of each sporocladium is always sterile, the last one produces a single conidium directly from the sharply curved apex or may be sterile. Should the conidiophores continue proliferation, more complicated forms would be evolved with typically more than two or three sporocladia arranged diffusely along the axis of the conidiophore. The majority if not all species of *Coemansia* develop in such a pattern.

Two problems are presented after consideration of *M. Corticii* and forms closely related to it—the first a question involving the generic position of this fungus—the second a question concerning the advisability of separating *Coemansia* from *Martensella*. Following Linder's interpretation of the two genera *Martensella* and *Coemansia sensu stricto*, *M. Corticii* is properly a *Coemansia*. This decision involved a simple consideration of the effect produced by the orientation of the host upon the fungus. In the final analysis it was seen that the phialides in nature actually are produced from the lower surface of the sporocladia.

The second problem is not dismissed so readily. *Martensella*, erected in 1863 by Coemans for a single species *M. pectinata*, now known only from the author's description, antedates *Coemansia* Van Tieghem & LeMonnier (1873) by ten years. Until the recent description of *M. Corticii*, it remained monotypic. The separation of the two genera as based on the orientation of the phialides may be artificial and may be conditioned by the position of the fungus on its substrate. In any species with a diffuse arrangement of the sporocladia the phialides, depending upon the angle of branching between the sporocladium and conidiophore axis, may be pointed in any direction. Such a nomenclatorial

problem is beyond the scope of this paper and for our present purpose it seems best to follow Linder's treatment. Future students of the group, however, may find it desirable to treat *Coemansia* as a synonym of *Martensella*, or perhaps to provide for the conservation of *Coemansia*.

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SOME LEAF SPOT FUNGI ON WESTERN GRAMINEAE—II ¹

RODERICK SPRAGUE ²

(WITH 2 FIGURES)

In addition to previous reports (6-12), more fungi that cause leaf spots, or are associated with diseased leaves, of grasses growing in the western United States are discussed or described in this paper. Type material and representative collections are filed in the Mycological Collections, U.S.D.A., Beltsville, Md. Some of the specimens are duplicated in the herbarium of the Dept. of Plant Pathology, Washington State College, Pullman, Wash., and in the Mycological Herbarium, Dept of Botany, Oregon State College, Corvallis, Ore. These studies have been made at the Northern Great Plains Field Station, Mandan, N. Dak.

Spermospora gen. nov.

Conidiis subulatis v. subulati-filiformibus, apicibus elongato-filiformibus, hyalinis, septatis, maculicolis.

Typus: *Cercospora subulata* Sprague.

Conidia subulate to subulate-filiform, apical cell appearing appendage-like; hyaline, septate, borne superficially on evident conidiophores in spots. A member of the Moniliales and the Moniliaceae.

¹ Cooperative investigations between the Divisions of Cereal Crops and Diseases, Forage Crops and Diseases, and Soils, Fertilizers and Irrigation Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration; and the Nursery Division, Soil Conservation Service, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

² Formerly pathologist, Division of Cereal Crops and Diseases, and collaborator, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering.

On the basis of continued study of a number of collections, the fungus *Cercospora subulata* (6) is made the type of the new genus. Accordingly, the name of the fungus becomes **Spermospora subulata** (Sprague) comb. nov. The diagnostic characters of *Spermospora* and *Cercospora* are as follows:

1. Conidia more or less broadly filiform, hyaline, septate—*Cercospora*.
2. Conidia subulate or narrow-subulate with distal cell elongated into a narrow whip-like tip, hyaline, septate—*Spermospora*.

Spermospora belongs, with *Cercospora*, in section *Scoleosporae* of the Moniliaceae (Moniliales). The sperm-like shape of the spores of *S. subulata* is so distinct that it seems desirable to separate this species from *Cercospora*.

Spermospora subulata was originally found on an herbarium specimen of *Melica subulata* (Griseb.) Scribn. from eastern Oregon (6). Although this fungus is definitely parasitic, causing severe leaf scald, it remains somewhat of a mystery why it is never abundant. It has been collected as far east as the Big Horn Mts., Wyo., by George W. Fischer, on *Melica bulbosa* Geyer (B.P.I. 80, 114). The writer found the same parasite on an alpine form of *Deschampsia caespitosa* (L.) Beauv. in Quad Creek meadow at Beartooth Pass, Mont. (B.P.I. 80,304), at an elevation of about 10,000 feet. In 1937 he also collected a small quantity of it on *Festuca rubra* L. west of Halsey, Ore., and near King's Valley east of Wren, Ore. John Hardison sent additional material from Granger, Ore., in 1945. This latter material, also on *F. rubra*, differs in some ways from the type. The spores (FIG. 1, A) are 3-septate instead of being typically 2-septate. The extra septum is usually formed at the base of the particularly appendage-like distal cell. This cell has the appearance of being partly germinated. The overall size of the spores is $33-55 \times 3.3-4.3 \mu$ whereas the type on *Melica* has spores $20-35 \times 2.5-4.3 \mu$ (6, FIG. 2, A). The collection on *Deschampsia* contains spores similar in size. They are faintly yellowish, 2-3-septate and $33-47 \times 3-4.5 \mu$ (FIG. 1, B). They are borne in scald-like obscure lesions, typical of the species.

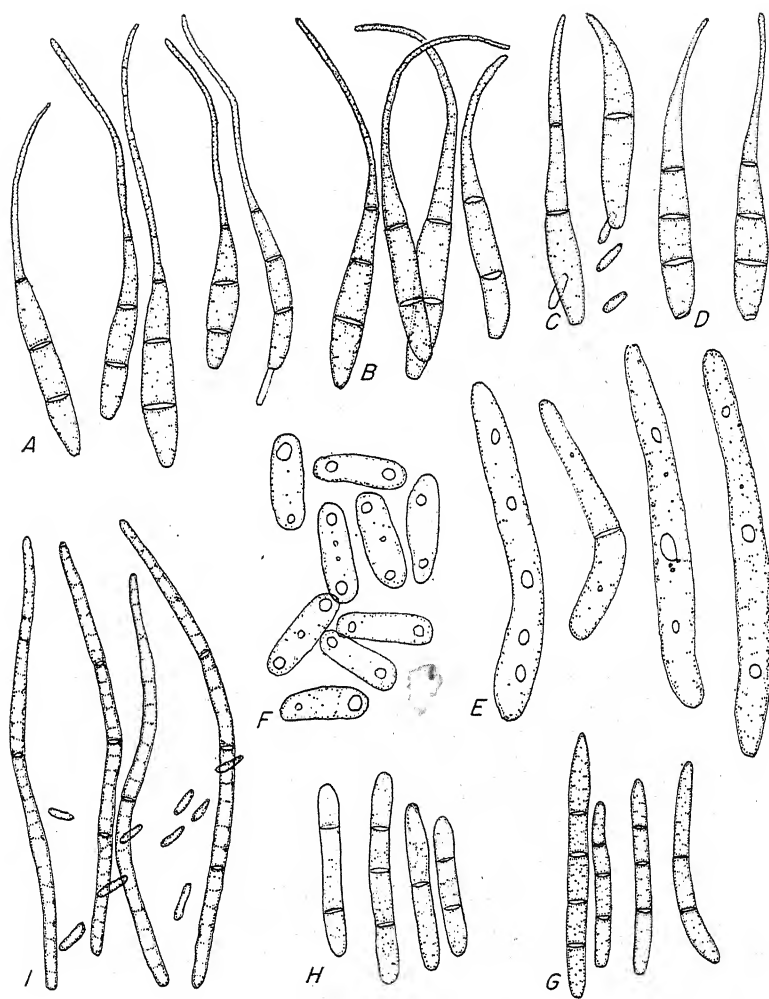


FIG. 1. Spores of leaf spot fungi on western grasses.

A pure culture was obtained from spores on *Festuca rubra* collected near Halsey, Ore., in 1937. The fungus grew slowly on potato-dextrose agar. The colony was felty, depressed, fawn colored and at first it was sterile. Later a few spores, macrospores and microspores, were produced in the refrigerator at 40° F., the macrospores (FIG. 1, C) being similar to those formed on the host (FIG. 1, D). The macrospores in pure culture measured $35-50 \times 3.4-4.6 \mu$ and the microspores, $4-6 \times 1.5-2.0 \mu$. The spores on the leaves of the Halsey collection measured $30-45 \times 3.0-4.6 \mu$. Those from both sources were 3-septate.

The spores in the 1945 Granger material sometimes retain the slender, elongate conidiophores. These evanescent appendages could be mistaken for basal cilia on the spores. They arise from compact hyaline to chlorinous mycelial masses. The conidiophore-bearing masses are strictly moniliaceous in nature and are not acervulus-like as in *Pseudodiscosia avenae* Sprague and A. G. Johnson (14).

On the basis of present evidence, all of the collections of *Spermospora* on the various genera of grasses belong in the one species, *S. subulata*.

Phleospora idahoensis sp. nov.

Maculis nigris, diffusis, pycnidiiis immersis, subglobosis v. poculiformibus, gregariis, brunneo-nigris, plerumque leptodermis, parenchymaticis, tarde ostiolatis, $90-150 \mu$ diam.; cirrhis brevibus, hyalinis; pycnosporulis clavato-cylindraceutis, rectis v. curvulis, apicibus subacutis, basibus obtusis, $0-1$ -septatis, hyalinis, $40-53 \times 4.6-5.6 \mu$.

Hab. in foliis et pedicellis vivis *Festucae idahoensis* Elmer, Trude, Idaho, July 28, 1945 (B.P.I. 81,145, *typus*). Legit George W. Fischer.

Spots black to dark brown, diffuse, pycnidia immersed, subglobose to cup-shaped, brown-black, gregarious, wall thin for the main part (12μ or less), parenchymatous, finally ostiolate or opening at the top through stomata, $90-150 \mu$; spore horns short but numerous, hyaline or faintly tinted; pycnosporules stoutly club-shaped to somewhat cylindrical, somewhat to distinctly curved, apices tapering, sometimes pointed, bases blunt but the spores narrowed towards the ultimate base, rarely 1-septate, usually aseptate, hyaline with coarse inclusions, $44-53 \times 4.6-5.6 \mu$ (FIG. 1, E).

There is considerable room for doubt as to whether this fungus belongs in *Phleospora* rather than *Septogloeum* on the one hand or

Septoria on the other. Before paraffin sections were made through the fruiting bodies the fungus appeared to belong in *Septogloeum* (13) but cross sections of the pycnidia indicate that it does not. The fungus shows strong resemblance to *Septoria infusans* (Ell. and Ev.) Sprague in the general shape of the spores and pycnidia. The presence of cirrhi is not always typical, the pycnidia are less well developed, have no, or only tardily formed, ostioles, and the spores are wider than the coarsest of the spores of *S. infusans*. These differences make it seem desirable to place it in a distinct species. Since the pycnidia are ill-formed, it appears to belong in *Phleospora*. This is borne out also by the coarse spores which are more or less typical of *Phleospora*.

Phleospora idahoensis is very distinct from *Septogloeum oxysporum* Sacc., Bomm. and Rouss. (7). It also is entirely different from *Cylindrosporium calamagrostidis* Ell. and Ev. which has spores $40-60 \times 1.5-2.0 \mu$. Although the spores of *P. idahoensis* have about the same dimensions as those of *P. graminivorum* Sprague and Hardison (2) they are very different in shape and in the number of septa. The pycnidia also are different.

Phleospora idahoensis derives its name from the specific name of its host and from the state of Idaho where it was found. Fischer collected it a short distance west of the Yellowstone National Park.

Ascochyta phleina sp. nov.

Maculis fulvellis, pycnidiis subgregariis, aureo-brunneis v. brunneis, globosis, erumpentibus, ostiolatis, $100-138 \mu$; pycnosporulis hyalinis, cylindraceis, utrinque rotundatis, 1-septatis, non constrictis, $11-16 \times 1.5-2.2 \mu$.

Hab. in foliis vivis et dejectis *Phlei pratensis* L. sociis *Helminthosporio* sp., *Cladosporio herbaro* Lk., *Heterosporio phlei* Gregory, *Hendersonia* sp. et *Xanthomonate* sp., prope Lake George, Niawa, Minn., June 4, 1941. Typus est B.P.I. 80,920.

Spots becoming tawny, pycnidia golden brown to brown, globose, strongly erumpent, more or less grouped, ostiolate, $100-138 \mu$; pycnosporules hyaline, cylindrical, both ends rounded with a tendency to be narrowed, contents with few small oil drops, nonconstricted at the one septum, $11-16 \times 1.5-2.2 \mu$, mean size $13 \times 1.75 \mu$, ratio of length to width 7.4 to 1.

This fungus appears to be moderately parasitic. It is sometimes associated with *Heterosporium phlei* and also with what appears

to be *Xanthomonas translucens* var. *phlei-pratensis* Wallin and Reddy on living leaves. In addition it is mingled with several other fungi on necrotic leaves. There had been some late frosts in the area and this contributed to the saprophytic development of some of the forms.

In final analysis this species is recognized because it does not fit any known species of *Ascochyta* on Gramineae. It is too small for the *A. graminicola* Sacc. group. It resembles a fungus on *Distichlis*, which is described in another paper in preparation (15), but the spores do not have the cylindrical or capsular shape of that species and in addition are hyaline, not yellow tinted. Morphologically, *A. phleina* may be close to *A. elymi* Tehon and Daniels but the latter species has cylindrical spores, $10-14 \times 2-3 \mu$ in pale brown pycnidia (16). The writer has seen this obscure species in small quantities a number of times and concludes that the species on *Phleum* is different from it not only because of the thin-walled pycnidia in *A. elymi*, but also because the spores of the latter are broader in the type. *A. phleina* has also been compared with *Septoria phleina* Baudys and Picb. (1). This has 3-septate spores similar to *S. triseti* Speg. (11). A few multiseptate spores were seen in mounts of *A. phleina* but they were cylindrical and not narrowly obclavate-filiform as in *S. phleina*.

An illustration of *A. phleina* is given in another article which deals exclusively with *Ascochyta* species on western grasses (15).

Phyllosticta healdii sp. nov.

Maculis diffusis, pallido-brunneis, concoloribus, pycnidiis nigris, numerosis, erumpentibus v. subsuperficialibus, ellipsoideis, plerumque in lineis dispositis, tarde ostiolatis, $111-156 \mu$ longis, $80-115 \mu$ latis; pycnosporulis hyalinis, bacillaribus v. capsuliformibus, aseptatis, medio contractis v. non constrictis, utrinque guttulatis, $11-15 \times 3.6-4.5 \mu$.

Hab. in foliis vivis et emortuis *Panici huachucae* Ashe, Valentine, Nebr. Typus est B.P.I. 81,192. June 15, 1946.

Spots covering most of infected leaves, diffuse, pale brown, of one uniform color, without margin, pycnidia black, more or less grouped in lines, erumpent to nearly superficial, ellipsoidal, mostly definitely longer than broad, $111-156 \mu$ long and $80-115 \mu$ wide, eventually ostiolate, ostiole small; spores in mounts usually need to be crushed from pycnidia, less often emerging through the ostiole; pycnospores hyaline, capsular to bacillar-shaped, non-septate

but many spores having a slight constriction or contraction at the centers; most spores with a prominent inclusion at each end with sometimes one or more smaller inclusions elsewhere, $11-15 \times 3.6-4.5 \mu$ (FIG. 1, *F*).

This species is characterized by the numerous black, linearly arranged pycnidia on diffuse brown leaf lesions and the short-cylindric "blocky" spores with their prominent guttulae at each end. The fungus occurs on living leaves and also on dying or dead leaves, some from the previous season. It is apparently perennial on the grass.

There appears to be no species to which this fungus can be referred. *Phyllosticta panici* E. Young (17, p. 144) has ovate spores, $4.8-9.6 \times 3.6 \mu$. We have identified *P. panici* on *Panicum virgatum* L. from material collected by Simon Wolff at Guthrie, Okla. and it is different from *P. healdii*. The spores of the latter are very much larger than those of *P. sorghina* Sacc. and are a somewhat different shape from those of *P. rogleri* Sprague on *Digitaria* in Iowa.

The fungus is named for Frederick D. Heald, major professor of the writer during his earlier student years. It was collected in Nebraska, one of the three states in which Dr. Heald founded modern departments of plant pathology.

Septoria glycericola sp. nov.

Maculis prominulis, semiorbicularibus, ochraceis, centro pallidis, pycnidiis aureo-brunneis, subglobosis, ostiolatis, $90-140 \mu$; pycnophoris papillatis; pycnosporulis lineari-cylindraceis, hyalinis, apicibus subacutis, basibus subobtusis, 1-3-septatis, $20-34 \times 1.9-3.4 \mu$.

Hab. in foliis et vaginis vivis *Glyceriae striatae* (Lam.) Hitchc. prope Hoover, Oreg. (**typus** est O.S.C. 10,177). Coll. H. P. Barss and G. B. Posey. Paratypes: *Glyceria striata*, White Hall, Ky. (J. H. Hardison); *G. canadensis* (Michx.) Trin., Dennis, Mass. (C. L. Lefebvre); *G. clata* (Nash) Hitchc., near Elgin, Oreg. (B.P.I. 80,113, G. W. Fischer); *G. grandis* S. Wats., Bismarck, N. Dak. (Laird Wolff, B.P.I. 81,188); *G. pauciflora* Presl, Cascade Mts., Wash. (Sprague); *ibid.*, Lawyer Canyon, Idaho (Sprague, Fischer, Meiners, C.S. 3679).

Spots prominent, buff, semiorbicular, becoming paler in the center, pycnidia scattered, golden brown, subglobose, slightly flattened, walls $6-7 \mu$ thick, composed of rectangular cells which finally collapse to a corky parenchymatous structure, pycnidia

90–140 μ diam., up to 90 μ tall; pycnophores absent, obscure, or short papillae, pycnosporos narrowly cylindrical, somewhat pointed to blunt at apex, rounded at the base and usually definitely blunter than at apex, hyaline, 1–3-septate, $20\text{--}34 \times 1.9\text{--}3.4 \mu$, mean size about $25\text{--}26 \times 2.8 \mu$ (FIG. 1, G).

This fungus was originally assigned to *Stagonospora glyceriae* Roum. and Fautr. but after studying more and better material it was very clear that it was not that species. Later (13) the fungus was placed tentatively in *Septoria nodorum* Berk. but with a notation that it might need to be classed as a distinct species. In studying a great mass of material of *S. nodorum* on a considerable number of hosts, the collections from *Glyceria* always constituted a distinct group because of their proportionately narrower spores with the pointed ends. The writer concluded that this was a case where his efforts to reduce the number of species of *Septoria* on grasses would only lead to confusion, and he has therefore followed the earlier suggestion of Lefebvre (personal letter) to describe the fungus as a new species.

Material collected July 10, 1946 at Bismarck, N. Dak., on *G. grandis* appears to be a summer stage with small spores in the pycnidia. The pycnidia, which are in faded spots, are minute ($60\text{--}80 \mu$), black, the spores 0–1-septate, $10\text{--}15 \times 1.3\text{--}1.6 \mu$, but have the characteristic pointed apex.

The material from Kentucky, sent by Hardison, has some of the spores blunter at the ends (FIG. 1, H) and hence closer to *S. nodorum* in shape but nevertheless still somewhat narrowed for that species.

SEPTORIA PASSERINII Sacc. on HYSTRIX PATULA Moench

Septoria microspora Ellis, with spores $6\text{--}12 \times 0.7\text{--}1.2 \mu$, was elsewhere referred to *S. passerinii* (11), being considered a microspore stage of that species. In 1945, material of *Septoria* spp. on *Hystrix patula* was collected at Cotton Lake, Minn. (B.P.I. 81,163). Most of the material has microspores $3\text{--}5 \times 0.5\text{--}0.8 \mu$ and macrospores $55\text{--}64 \times 1.8\text{--}2.4 \mu$ (FIG. 1, I) borne in black, carbonaceous, erumpent, globose pycnidia, $120\text{--}205 \mu$ in diameter. The macrospores are filiform, somewhat pointed at the apex, less

pointed at the base. They are faintly 3-septate, hyaline with small hyaline inclusions. The spores are proportionately longer than the general concept of the stubby spored *S. passerinii*, which usually has macrospores $22-45 \times 1.5-2.2 \mu$. However as reported earlier (11), overwintering material on *Hordeum nodosum* L. has spores as large as $56 \times 2.7 \mu$. We saw somewhat similar material on *Hordeum distichon* L. at Sundance, Wyo., in June, 1946. It appears conservative therefore to include the *Hystrix* fungus in *S. passerinii*.

In addition to *S. passerinii*, the collections from Cotton Lake contain a smaller amount of a species of *Septoria* with 3-septate spores, $29-33 \times 2.5-3.1 \mu$. These spores (FIG. 2, A) are cylindrical, very distinct from *S. passerinii* and somewhat intermediate in size between *S. nodorum* Berk. and *S. avenae* Frank. The fungus may be similar to a species on wheat, *Elymus* spp., and *Agropyron* spp. in the western United States and Eurasia which has not been satisfactorily classified as yet. The fungus may belong in this group, which is typified by *S. avenae*. However, it seems to the writer that it is somewhat less robust than *S. avenae* and can better be placed in *S. nodorum* at this time. In this connection, a common, purple to fuscous blotch on the marsh grass *Fluminea festucae* (Willd.) Hitchc. appears to belong in *Septoria avenae* (FIG. 2, B). The writer had assigned this to his polymorphic concept of *Stagonospora arenaria* Sacc. but the spores in a majority of the collections from North Dakota and South Dakota are close to *S. avenae*, averaging about $27-33 \times 2.4-3.0 \mu$. Most of them are 1-septate summer spores. Therefore, although they average about the same size as those of the fungus on *Hystrix*, the different environment indicates that they are likely to prove larger than that under later summer conditions. The species of this entire group, ranging from the small *S. nodorum* to the somewhat larger *S. avenae* to the similar but still coarser *St. arenaria*, are difficult to separate in many cases. There are strong indications that all three, if they are all distinct, are not confined to any particular group of hosts, although *St. arenaria* appears to be commonest on *Elymus* and *Agropyron*, *S. avenae* on oats and oat relatives, and *S. nodorum* occurs on many grasses. One solution to this problem may be through a detailed study of the ascigerous stage of *S. avenae*.

(*Leptosphaeria avenaria* G. F. Weber), which may occur on other hosts than oats.³

SEPTORIA QUINQUESEPTATA Sprague on KOELERIA CRISTATA
(L.) Pers.

Septoria quinqueseptata was obtained on *Koeleria cristata* in the plots at Mandan, N. Dak., on Aug. 28, 1943 (B.P.I. 80,871). At that time the spores were obscurely 3-septate, $38-55 \times 2.2-2.7 \mu$. Two years later when re-examined, they had become very clearly 5-7-septate, mostly 5-septate. It is not unusual to find that viable herbarium material continues to develop for a time after collecting and before desiccation checks further changes. In 1943, the spores in pure culture on potato-dextrose agar were $38-55 \times 1.9-2.4 \mu$ (FIG. 2, C, a). The colonies were cottony to sub-cottony and were rosy buff in color.

Septoria quinqueseptata on *Koeleria cristata* differs from the common *Septoria calamagrostidis* f. *koeleriae* (Cocc. and Mor.) Sprague in several ways. The spores of the latter are much narrower, $35-82 \times 1.1-2.1 \mu$ (FIGS. 2, C, a and b; 2, D). This species also produces a mucose or wet yeasty growth in pure culture, with rosy buff cottony mycelium formed only after the colonies begin to stale in old cultures (11).

A comparison of the Mandan material on *K. cristata* with the type of *Septoria quinqueseptata* on *Sphenopholis obtusata* (Michx.) Scribn. (11) convinces the writer that it belongs in that species. The spores of this fungus in both the type and on *K. cristata* are too narrow and too short for *S. andropogonis* f. *sporobolicola* Sprague (9, FIG. 1, A).

Some confusion existed in the determination of the host of the Mandan material. *Koeleria cristata* was growing intermixed with the rhizomatous species of grass, *Calamagrostis montanensis* Scribn., which was rapidly crowding out *K. cristata*. As the two species are superficially similar, careful check was later made to determine if the host was not actually *Calamagrostis*. It was de-

³ Since our paper went to the printer a long anticipated, enlightening article by T. Johnson has appeared: A form of *Leptosphaeria avenaria* in Canada. Canad. Journ. of Research 25 (6): 259-270.

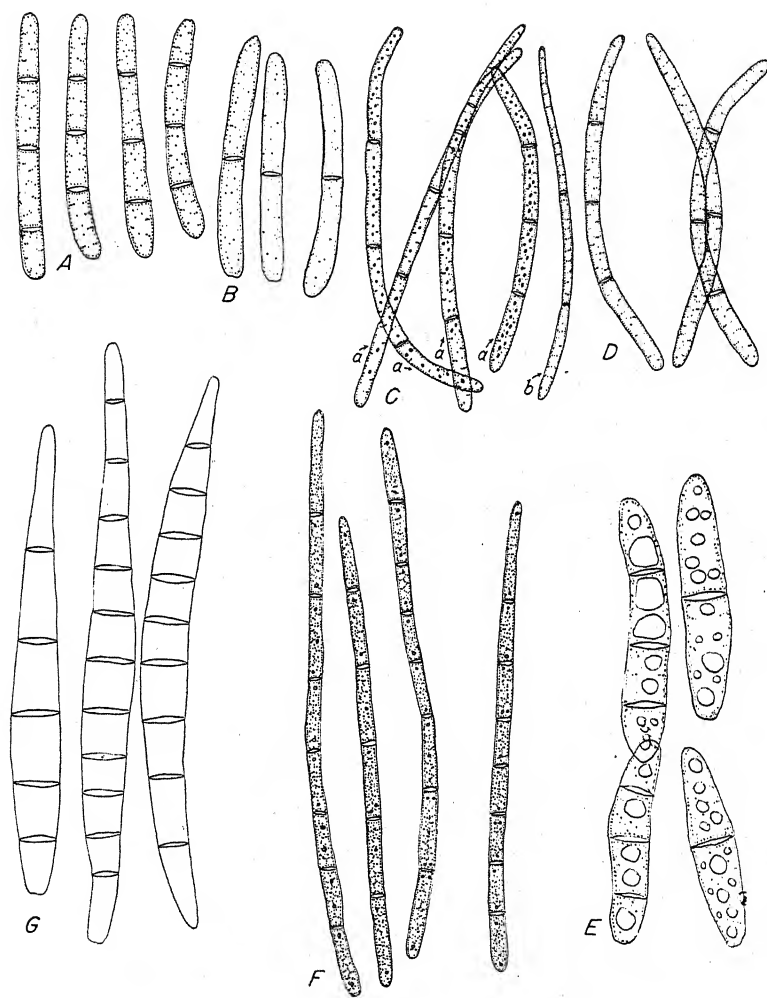


FIG. 2. Spores of leaf spot fungi on western grasses.

terminated beyond a doubt from fruiting material of the host, however, that the fungus in question was growing on *K. cristata*.

Septoria quinqueseptata is doubtfully parasitic on *Koeleria cristata* and possibly represents an accidental development on this grass. Apparently it does not occur on this host at all commonly.

SEPTORIA TANDILENSIS Speg.

This species, which was discussed recently (12, p. 54), has since been collected on *Panicum capillare* L. at Mandan, N. Dak., August 13, 1946. The collection was made a few hours after a hard rain which broke a long drought. The spores, which were typical otherwise, had evidently taken up moisture and started to germinate *in situ*. Some were swollen and as large as $111 \times 2.7 \mu$, others were normal size. The latter were $50-80 \times 1.3-1.6 \mu$ with all gradations between that size and the swollen ones. The curved, hyaline, faintly multiseptate spores, except in swollen ones, were borne in typical black pycnidia in small black, drought-inhibited spots. In spite of the aberrant aspect of the spores, this fungus is unquestionably *S. tandilensis*. This is the first report of it in the plains area of North Dakota and represents nearly a thousand miles extension of its range westward from Wisconsin, where it is well known.

SEPTORIA CENCHRINA J. J. Davis

This species was collected on *Cenchrus pauciflorus* Benth. at Mandan, North Dakota, Aug. 14, 1946, an extension of its range from Minnesota into the plains country. It produced pale obscure pycnidia on yellowed leaves without appreciable spot formation although some lesions were delimited linearly by the leaf veins. No doubt the fungus is commoner than collections show because the symptoms are very obscure.

Pure cultures were readily obtained on potato dextrose agar. The light-cream colored juvenile colonies developed, at room temperature, into black mounded carbonaceous growths from which minute spore masses exuded. The spore masses did not run together but remained separate as flesh colored to dirty pink pin

points over the black carbonaceous mass of the culture. The cultures were more vigorous than those of *S. infuscans* but tended towards a similar type of growth rather than like the mucose colonies produced by most species of *Septoria* on grasses. The spores in pure culture were typically long and straight-sided without appreciable difference in dimension from one end to the other. They were faintly 3-7-, mostly 5-7-septate under the most critical illumination. This is interesting because it had not been determined just how many septa, if any, spores of this species had. The septa were readily seen with the N. A. 1.32 lens. The contents were opaque under critical illumination but with a lens with a longer focal distance, N. A. 1.15, which the writer frequently uses, the spores appeared faintly yellowish. Some of them were as large as $125 \times 2.4 \mu$.

Stagonospora glycericola sp. nov.

Maculis nullis v. stramineis, pycnidiiis globosis, erumpentibus, non gregariis, aureo-brunneis, $90-140 \mu$ diam.; pycnosporulis subcylindraceis, v. subfusiformibus, constrictis v. non constrictis, apicibus et basibus subobtusis, hyalinis, globulatis, 1-3-septatis, $22-30 \times 4.6-6.4 \mu$.

Hab. in foliis languidis *Glyceriae striatae* (Lam.) Hitchc., Niawa, Minn. **Typus** est B.P.I. 81,180 et paratypus in *G. grandis*, Edinburg, N. Dak. (B.P.I. 81,189).

Spots none to straw colored, pycnidia globose, erumpent, non-gregarious, golden-brown, $90-140 \mu$ diam.; pycnosporules subcylindrical to subfusiform, constricted to non constricted, apices and bases subobtuse, hyaline, globulate, 1-3-septate, $22-30 \times 4.6-6.4 \mu$.

On sickly leaves of *Glyceria striata* (Lam.) Hitchc., Minn.

This fungus (FIG. 2, E) might be referable to *St. subseriata* (Desm.) Sacc. except that the spores of the latter are so much more fusiform or even boat-shaped (8, FIG. 1, B, D). In shape it is possibly closer to *St. subseriata* var. *maculata* Grove (8, FIG. 1, A) but this active parasite of *Dactylis glomerata* L. differs in many ways from the fungus on *Glyceria*. Incidentally, the writer considers that *St. subseriata* var. *maculata* Grove deserves specific rank, and accordingly *Stagonospora maculata* (Grove) comb. nov. is proposed. This fungus is becoming recognized as the cause of an important leaf disease of orchard grass, especially in pastures in the Middle Atlantic states.

A search of available species of *Stagonospora* does not disclose any on grasses that are similar enough to *St. glycericola* to justify assigning the two collections on *Glyceria* to a previously described species. According to description alone, *St. glyceriae* Roum. and Fautr. is closer to *Ascochyta graminicola* var. *brachypodii* Trail than to our fungus. The 1-septate spores of *S. glyceriae* are $16 \times 4 \mu$. Those of *A. graminicola* var. *brachypodii* are $22-25 \times 5.6-6.2 \mu$. Thus the evidence is against *St. glyceriae* being a phase of *St. glycericola*, and so they are considered distinct. Another species, *St. glyceriae* Kupka (4, p. 162), is even more distinct from *St. glycericola*. *St. glyceriae* Kupka has non-hyaline spores, $50-80 \times 3-4 \mu$, with 7-11 septa, all of which means that it probably belongs in the genus *Phaeoseptoria*, probably *P. festucae* Sprague (10), which has spores $50-85 \times 2.8-4.8 \mu$ with 8-11 septa. Incidentally we have collected *P. festucae* on *Glyceria grandis* in North Dakota although another collection (B.P.I. 80,072) from Pingree, N. Dak., was finally assigned to *Hendersonia crastophila* Sacc. because the spores were too short for *P. festucae* Sprague.

In considering graminicolous species of *Stagonospora* not on *Glyceria*, except for *St. subseriata*, there are few species of this genus in the 3-septate group with spores within the size range of *S. glycericola*. One of these is *S. alopecuri* Rostr. It has spores $25-32 \times 5-6 \mu$ but the pycnidia are listed as gregarious and prominent and the spores are bacillar-cylindrical, none of these characters being at all like those of *St. glycericola*. *Stagonospora brachypodii* Died. answers the description of *S. glycericola* very well except that the darker pycnidia and yellow spores indicate, as Saccardo says (5, v. 25, p. 367), that the fungus is close to *Hendersonia culmicola* f. *minor* Sacc., which is different from ours.

Phaeoseptoria poae sp. nov.

Pycnidiis nigris, carbonaceis, globosis, subsuperficialibus, $150-220 \mu$, ostiolatis; pycnosporulis chlorinis, filiformi-clavulatis, apicibus acutis, $50-77 \times 2.1-2.4 \mu$, 5-7-septatis.

Hab. in foliis emortuis *Poa canbyi* (Scribn.) Piper (*P. lucidae* Vasey). Custer National Forest, prope Ekalaka, Mont., June 21, 1945 (B.P.I. 81,144).

Pycnidia black, fragile or brittle, carbonaceous, globose, sub-superficial, 150–220 μ , ostiolate; pycnospores yellow, filiform-clavulate, pointed at the apex, slightly blunted at the base, 50–77 \times 2.1–2.4 μ , 5–7-septate (FIG. 2, F).

Habitat in dead leaves of *Poa canbyi* in the hills in open scrub woods, Custer National Forest near Ekalaka, Mont.

Phaeoseptoria poae differs from *P. airae* (Grove) Sprague in having nearly filiform spores with 5–7 septa, whereas *P. airae* has wider spores with 9–10 or even 13 septa (10, FIG. 1, G).

The host in the type material of *P. poae* appears to be a pale green form of *Poa canbyi* sometimes known as *Poa lucida* Vasey, which Hitchcock reduces to synonymy (3).

A collection of *Phaeoseptoria festucae* was made on *Poa nevadensis* Vasey at Mandan, N. Dak. (B.P.I. 80,866). This species has distinctly broader spores than *P. poae*. *P. festucae* appears to be widespread but a minor saprophyte on a number of grasses. It has been collected on *Festuca rubra* and *Danthonia parryi* Scribn. as well as on *Glyceria grandis* mentioned earlier in the article. A form of it has been found on *Muhlenbergia mexicana* (L.) Trin. (10). This same form occurs on *Elymus virginicus* L. in North Dakota. A form on *Andropogon furcatus* Muhl. with very coarse spores (FIG. 2, G) is being described separately as a new variety of *P. festucae*.⁴

The writer wishes to thank Dr. A. G. Johnson, Miss Edith K. Cash and Mr. John A. Stevenson for aid in the preparation of this and earlier papers. The mycological notes issued by the writer from time to time during the past 18 years have been a by-product of more pressing studies on diseases of Gramineae. They have been offered because there was need for even incomplete data, such as these, on the leaf spots of cereals and grasses. There is a great deal to be done in the way of critical life history studies in this group.

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⁴ The description will be included in a paper by H. C. Greene, the fungus having been collected by him in Wisconsin and sent to the writer for determination.

EXPLANATION OF FIGURES

FIG. 1. *A*, conidia of *Spermospora subulata* on *Festuca rubra*, Granger, Ore., 1945; *B*, conidia of *S. subulata* on *Deschampsia caespitosa*, Beartooth Pass, Mont.; *C*, conidia, macrospores, and microspores, of *S. subulata* in pure culture on potato dextrose agar from *Festuca rubra*, west of Halsey, Ore., 1937; *D*, conidia of *S. subulata* on *F. rubra*, west of Halsey, Ore., 1937; *E*, pycnosporos of *Phleospora idahoensis*, type; *F*, pycnosporos of *Phyllosticta healdii* on *Panicum huachucae*, ex type; *G*, pycnosporos of *Septoria glycericola* on *Glyceria canadensis*, Dennis, Mass.; *H*, pycnosporos of *S. glycericola* on *Glyceria striata*, White Hall, Ky.; *I*, pycnosporos of *S. passerinii* on *Hystrix patula*, Cotton Lake, Minn. (all $\times 1000$).

FIG. 2. *A*, Pycnosporos of *Septoria nodorum* on *Hystrix patula*, Cotton Lake, Minn.; *B*, pycnosporos of *Septoria avenae* Frank on *Fluminea festucaeae*, Sand Lake, S. Dak.; *C*, *a* pycnosporos of *Septoria quinqueseptata* from pure culture (potato-dextrose agar) isolated from *Koeleria cristata*, Mandan, N. Dak., compared with *b* pycnosporos of *S. calamagrostidis* f. *koeleriae* from Corvallis, Ore.; *D*, pycnosporos of *Septoria quinqueseptata* on *Koeleria cristata*, Mandan, N. Dak.; *E*, pycnosporos of *Stagonospora glycericola*, ex type; *F*, pycnosporos of *Phacoseptoria poae*, ex type; *G*, pycnosporos of *P. festucae* var. *ined.* (all $\times 1000$).

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HYPHAL PROLIFERATION THROUGH CLAMP-FORMATION IN POLYPORUS CINNABARINUS FR.¹

JOHN B. ROUTIEN²

(WITH 1 FIGURE)

Though Kniep (3, figs. 9 and 10 and 4, fig. 2) and Bensaude (1, fig. 18) long ago illustrated in certain Agarics the origin of some branches of the hyphae from clamp-connections, this behavior appears to be mentioned rarely in mycological literature. Drechsler (2) also has illustrated the development of branches from clamp-connections in *Nematogonus haptocladus*, a fungus parasitic on nematodes. In 1936 Rogers (5) described in a new *Sebacina* a process of repeated branching of the clamp-connections basal to the basidia. He stated (5), "The binucleate apical cells . . . develop into basidia, the second basidium being borne on the clamp at the base of the first. By repetition of this process a unilaterally cymose cluster of basidia is formed."

Casual examination of the hyphae of certain tissue-cultures of sporophores of *Polyporus cinnabarinus* Fr. revealed that many branches developed from clamp-connections, and it seemed desirable to describe the formation of these branches in detail.

Tissue-cultures from two sporophores growing on the same log, but about 15 ft. apart, showed abundant branching from the clamp-connections. Another culture from a sporophore collected about one-fourth mile from the afore-mentioned specimens, however, showed no branches arising from clamps.

Twenty single-basidiospore cultures derived from one of the first-mentioned sporophores were paired in all possible combinations and examined microscopically. Branches developing from

¹ Contribution from the Mycology Section, Chas. Pfizer & Co., Brooklyn, N. Y.

² The author acknowledges gratefully the technical assistance of Miss Dorothy E. Meyn.

clamp-connections were seen in nearly all of the dicaryon cultures that developed from these matings. Presumably, closer examination would have revealed their presence in all pairings.

To determine the nuclear behavior in these branches arising from clamp-connections, transfers of one of the tissue cultures were grown on a small amount of potato-dextrose-agar on a sterile slide in a damp chamber at 28° C. for 3-4 days. The specimens then were killed and fixed in strong chrom-acetic acid mixture and stained with Harris' Hematoxylin (6). Nuclear figures of the stained slides are interpreted in the following manner.

The dicaryon condition of the cells of the culture is maintained through the generation of clamp-connections accompanying conjugate nuclear division. Branches may develop near the terminal end of a cell at some point other than from a clamp-connection, but apparently a clamp-connection develops very early in the ontogeny of such a branch in the culture used.

More frequently, however, in this particular culture, a branch will arise from a very young (FIG. 1, *a*) or moderately older clamp-connection and will grow out at an acute or obtuse angle to the main hypha. At first the nuclei of the cell subtending the clamp-connection are in about the middle of the cell (FIG. 1, *b*), but very soon after initiation of the branch they may be found near the clamp-connection (FIG. 1, *a*). A little later one of the nuclei will be seen in the branch with the second nucleus apparently about to enter (FIG. 1, *c* and *d*). When the branch is only slightly longer two nuclei may be seen in it, whereas the cell subtending the clamp-connection shows no evidence of nuclear material (FIG. 1, *e*). The next stage found is that in which the two nuclei are undergoing conjugate nuclear division (FIG. 1, *f*). This is followed by the formation of a short branch directed away from the tip of the cell with one of the daughter nuclei in its tip, one in the cell of the main hypha and two in the branch (FIG. 1, *g*). This short branch can be seen to fuse with the cell of the main hypha (FIG. 1, *h*) at about the time that septa delimit the new dicaryon cell from the original cell (FIG. 1, *h*). Sometimes, however, these septa do not form so soon (FIG. 1, *i*). The final condition, then, can be seen in figure 1, *j*.

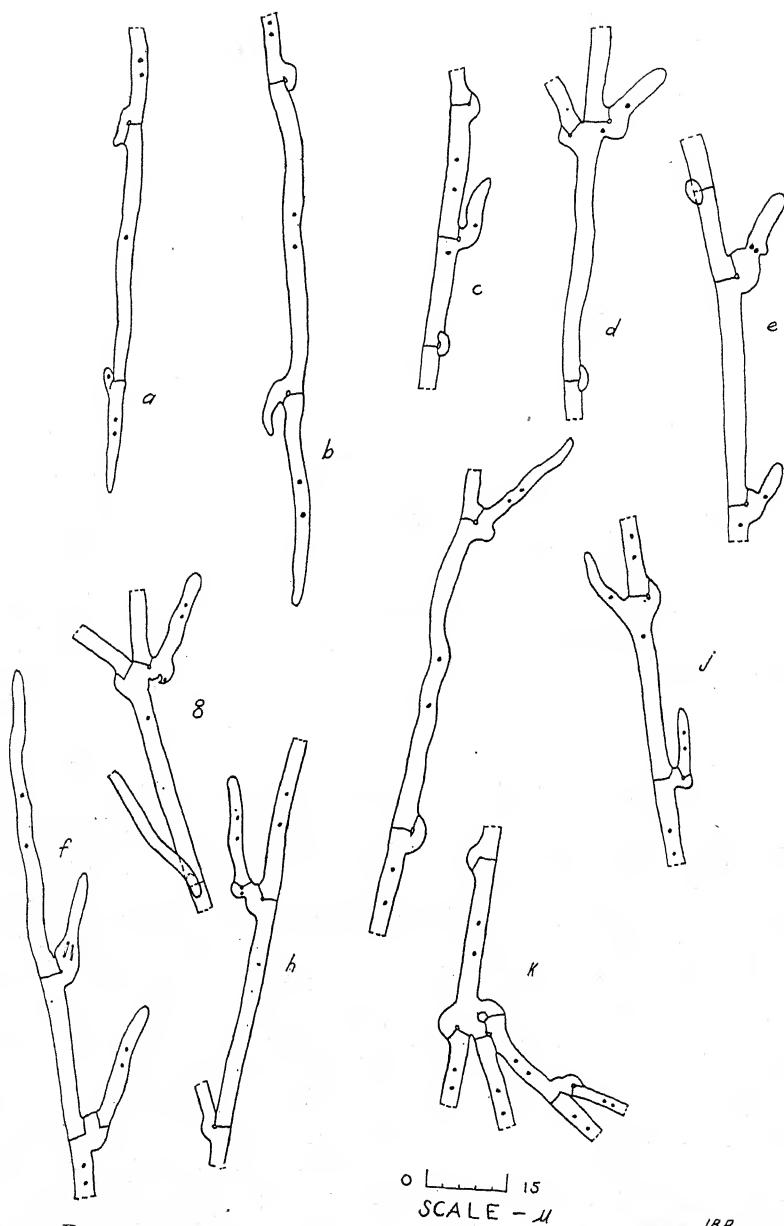


FIG. 1. Development of hyphae from clamp-connections in *Polyporus cinnabarinus*.

Much more complex figures are not uncommonly found; figure 1, *k* represents such a system. This particular figure can be explained as follows: opposite the clamp-connection on the main hypha a branch began to develop that immediately formed a large clamp-connection. From the surface of this clamp-connection another branch and clamp-connection began to develop simultaneously. Septa cutting off the clamp-connections from the cells are oriented peculiarly because the two clamp-connections are so near each other.

The nuclear behavior described here seems to be the same as that described by Kniep (3) for *Corticium varians* and (4) for *Collybia conigena*, by Bensaude (1) for *Tricholoma nudum* and by Rogers (5) (for basidial proliferation) in a *Sebacina*.

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EXPLANATION OF FIGURES

FIG. 1. *Polyporus cinnabarinus* Fr. Free-hand drawings made from preparation examined at approximately 1200 magnification with compensating eye-pieces. Only nuclear contents of cells are shown. *a*. Terminal portion of a hypha showing very young branch developing from a clamp-connection with nuclei of ante-penultimate cell near the clamp-connection; also shown is a nearly complete clamp-connection in the terminal cell. *b*. Slightly longer branch developing from clamp-connection; nuclei involved still are in the middle of the cell. *c*. Short branch from a clamp-connection; one nucleus has entered the branch, while the second is about to enter. *d*. Slightly more

advanced stage. *e.* Stage in branch development in which both nuclei have entered the branch; note start of formation of new clamp-connection. *f.* Conjugate nuclear division of the two nuclei in the branch. *g.* Further development of clamp-connection. *h.* New clamp-connection is completely formed; new branch has two nuclei, and parent cell still is dicaryon. *i.* New branch is formed with two nuclei and parent cell has two nuclei; septa separating the two "cells" have not formed. *j.* Final stage in which both nuclei of the parent cell have returned to about the middle of the cell. *k.* Structure produced by development of branches and clamps in rapid succession.

THE BOLETES OF YUNNAN¹

WEI FAN CHIU²

In the latter part of the nineteenth century foreign explorers such as Potanin and others who collected higher plants in this country incidentally picked up fungi at the same time (Tai: Sci. Rept. Nat. Tsing Hua Univ. Ser. B 2: 137-139, 1936). Of their fungus collections two new genera and ten species (including three new) of the Boletaceae were reported (Patouillard: Bul. Soc. Myc. Fr. 11: 106, 1895; Kalchbrenner: Bul. Acad. Imp. Sci. St. Peterb. 27: 135, 1881; Lohwag: Simbolae Sinicae 2: 54, 1937). Since 1932, Teng carried on a study of the fleshy fungi of China, and recorded, up to 1938, twenty-three species of this group, including one new species (A Contr. to Our Knowledge of the Higher Fungi of China. Acad. Sinica, 1939). In the present paper fifty-four species and two varieties, including twenty-two new species and one new variety, are recorded. All of the specimens, except a few, were collected in Yunnan in the Southwest of China.

BOLETINUS Kalchbrenner

Boletinus kunmingensis sp. nov.

Pileo 3-5 cm. lato, pulvinato dein convexo, "Baryta Yellow,"³ aetate "Tawny," viscido, glabro; tubulis 3-4 mm. longis, "Pinard Yellow" v. "Buff Yellow," decurrentibus; poris majusculis, plus minus hexagonalibus v. alveolatis, radiatim dispositis; stipite 3-4 cm. longo, 3-7 mm. crasso, "Baryta yellow," subaequali v. deorsum attenuato, fusco-punctato; carne flava. Spor. pallide olivaceis, 9-11 × 4-5 μ (10 × 5 μ).

¹ Paper No. 17 from the Department of Plant Pathology, College of Agriculture (formerly Institute of Agricultural Research), National Tsing Hua University, Peiping, China.

² This work was done under the direction of Prof. F. L. Tai to whom the writer wishes to express his indebtedness for encouragement, valuable suggestions and help in the preparation of the manuscript. Thanks are also due to Dr. W. H. Snell of Brown University for reading the manuscript and making valuable suggestions.

³ Refer to Ridgway, R. Color Standards and Color Nomenclature. Washington, D. C., 1912.

Pileus 3-5 cm. in diameter, at first pulvinate to hemispherical, becoming convex, "Baryta Yellow" when young, "Tawny" with age, very viscid when moist, glabrous; margin wavy. Tubes 3-4 mm. long, short-decurrent, "Pinard Yellow" or "Buff Yellow"; pores large, hexagonal, radially arranged, dotted with glandules. Stipe 3-4 cm. long, 3-7 mm. thick, "Baryta Yellow," subequal or tapering downward, dotted with dark-colored granules, stuffed. Flesh yellowish, paler in the cap.

Spores pale olivaceous under the microscope, elliptical, $9-11 \times 4-5 \mu$ ($10 \times 5 \mu$).

Nov. 12, 1941, W. F. Chiu, Tiehfungan, Kunming (7695),
Type.

This fungus is close to *B. punctatipes* Snell et Dick, but differs from the latter in its larger spores. It has the same habitat as *B. punctatipes* Snell et Dick var. *pinetorum*, but it differs from the latter in the entirely punctate and yellow-colored stipe.

BOLETINUS PUNCTATIPES Snell et Dick var. *pinetorum* var. nov.

A typo differt stipite semper subconcolore nunquam albido, apice non reticulato.

Pileus 4-9 cm. in diameter, hemispherical, becoming broad convex to plane, "Flesh Ochre," "Cinnamon Rufous" or "Orange Cinnamon," very viscid when moist, glabrous; margin wavy. Tubes 3-5 mm. long, light "Cadmium Yellow," decurrent; pores large, $3-4 \times 1-2$ mm., compound, radially arranged, concolorous with the tubes, dotted with glandules. Stipe 2-5 cm. long, 5-15 mm. thick, subconcolorous with the cap but usually paler, yellowish at the apex, minutely punctate at the upper part of the stipe with brown dots, rarely slightly reticulate at the apex, tapering downward. Flesh white or pinkish in the cap, yellowish adjacent to the tubes and in the stipe, "Orange Yellow" at the basal part of the stipe.

Spores pale olivaceous under the microscope, elliptical, $6-11 \times 3-4 \mu$ ($8 \times 3 \mu$).

June to Aug. 1940, F. L. Tai and C. C. Cheo, Tapugi, Kunming (7888 and 7725); Tiehfungan, Kunming, Nov. 12, 1941, W. F. Chiu (7717 **Type**, 7720 and 7730); Aug. 1938, C. C. Cheo, Tali (7691).

This variety differs from var. *typica* in that its stipe is never white or whitish but instead concolorous with the cap and that

the reticulation at the apex of the stipe is rare to occasional. The American plant occurs commonly under firs whereas the Yunnan form is commonly associated with pines (*Pinus yunnanensis*).

BOLETINUS PICTUS Peck

Pileus 2–13 cm. in diameter, hemispherical, convex becoming plane, dry, "Pompeian Red" or "Morocco Red," at first paler, then darker, covered with adpressed fibrils and scales; scales separated in later development, and becoming "Brussels Brown"; "Light Cadmium" yellow between and beneath the scales. Tubes 3–5 mm. long, "Light Cadmium" yellow, decurrent; pores 1–2 mm. across, concolorous with the tubes, becoming ochraceous with age, radially arranged, angular, compound. Stipe 6–12 cm. long, 8–35 mm. thick, yellow above the ring, "Light Buff" or "Warm Buff" below, tinged "Morocco Red" in the lower portion, coarsely reticulate or streaked, sometimes fibrous, turning reddish-brown with age, subequal or ventricose at the base; ring grayish, membranous or rather cottony. Flesh "Pale Yellow Orange," pinkish in the stipe.

Spores olivaceous under the microscope, elliptical, $9-11 \times 3-4 \mu$ ($9 \times 4 \mu$).

Aug. 24, 1938, C. C. Cheo, Chungotze, Tali (7782, 7783 and 7781); July 23, 1938, C. C. Cheo, Chenkung market (7890); Aug. 12, 1942, W. F. Chiu, Shishan, Kunming (7880 and 7881). Usually under *Pinus Armandii*.

The typical forms of this species were collected under pines in the vicinity of Kunming, but variation was noted in that the squamules of the cap usually change to "Brussels Brown" in the later stage of development, or are of such a color even when young. The stipe is not always cylindrical but sometimes bulbous. The fibrillose and adpressed scales may be also present on the stipe in certain cases.

BOLETUS Fr.

Boletus minutus sp. nov.

Pileo 1–3 mm. lato, carnoso, pulvinato dein convexo, glabro, "Sudan Brown"; tubulis usque ad 0.5 mm. longis, ochraceis, decurrentibus; poris imperfectis, daedaloides, griseo-brunneis; stipite 3–4 mm. longo, 0.6–1 mm. crasso, subaequali, leviter ventricoso v. sursum attenuato, pileo concolore, glabro, farcto; carne brunneola, immutabili; sporis olivaceis, ellipsoideis, $4.5-7 \times 2-2.5 \mu$ ($6 \times 2.5 \mu$).

Pileus 1-3 mm. in diameter, pulvinate to convex, glabrous, "Sudan Brown." Tubes up to 0.5 mm. long, ochraceous, short-decurrent; pores daedaloid, 0.1-0.2 mm. across, grayish brown. Stipe 3-4 mm. long, 0.6-1 mm. thick, slightly ventricose or tapering upward, concolorous with the cap but paler, glabrous, stuffed. Flesh brownish, unchanging.

Spores olivaceous under the microscope, elliptical, $4.5-7 \times 2-2.5 \mu$ ($6 \times 2.4 \mu$).

June 25, 1942, Miaokaotze, Kunming, W. F. Chiu (8041), **Type.** On mossy slope in mixed woods.

This plant is outstanding for its minute stature. Its hymenophore, as far as observed under the microscope, does not uniformly consist of tubes with round pores. Some tubes possess a somewhat daedaloid orifice. Furthermore, its typical small spores are also rare in boletes. At present, it is tentatively placed in the genus *Boletus*.

BOLETUS CASTANEUS Fr. ex Bull.

Pileus 4-6 cm. in diameter, convex to plane, "Russet Brown" to "Amber Brown," minutely tomentose. Tubes short, about 2.5-3 mm. long, at first white then yellowish, free from the stipe; pores less than 1 mm. across, whitish. Stipe 5-8 cm. long, 1-2 cm. thick, concolorous or paler than the cap, minutely tomentose, subequal, stuffed. Flesh white, unchanging.

Spores hyaline or subhyaline under the microscope, short ellipsoid, $8-9 \times 5-6 \mu$ ($8 \times 5.5 \mu$), rarely reaching 10μ long.

July 13, 1938, C. C. Cheo, Chunchutze, Kunming (7884); Aug. 28, 1938, C. C. Cheo, Tali (7702 and 7806).

BOLETUS FLAVIDUS Fr.

Pileus 3-7.5 cm. in diameter, semiglobose to convex, glabrous, "Sayal Brown." Tubes up to 4 mm. long, white; pores small, roundish, free. Stipe 2-3.5 cm. long, 1-1.5 cm. thick, concolorous with the cap, glabrous, subventricose. Flesh white, unchanging.

Spores hyaline under the microscope, oblong-elliptical, $3.5-5 \times 5-7.5 \mu$.

July 27, 1939, W. F. Chiu, Mt. Omei, Szechwan (Chiu 135).⁴

⁴ Specimens collected outside Yunnan and deposited in the Herbarium of the Plant Pathology Department, University of Nanking, Nanking, China.

Boletus atrovioleaceus (v. Höhn.) comb. nov. (*Suillus atrovioleaceus* v. Höhn. Frag. zur Myc. n. 835 in Sitzgsb. Akad. Wiss. Wien CXXIII (1): 87, 1914).

Pileus 2.6 cm. in diameter, plane to convex, "Bluish Violet," tinged "Tawny" at the margin, minutely tomentose. Tubes short, 3 mm. long, white, free from the stipe; pores 0.2 mm. across, white, turning reddish-brown with age, angular, simple. Stipe 3.5 cm. long, 4-9 mm. thick, concolorous with the cap above, "Tawny" below, granulose-tomentose in the upper portion, subventricose in the lower part, hollow. Flesh whitish.

Spores hyaline under the microscope, short-ellipsoid, $8-11 \times 6-8 \mu$ ($9 \times 6 \mu$).

July 16, 1938, C. C. Cheo, Shishan, Kunming (7901).

BOLETUS FLAVUS With.

Pileus 2-6 cm. in diameter, convex, very viscid when moist, shining when dry, "Pinard Yellow" to "Empire Yellow," slightly rugose with age. Tubes up to 6 mm. long, "Aniline Yellow," becoming "Orange Citrine," compound, adnate decurrent; pores up to 2 mm. across, somewhat smaller near the marginal region, concolorous with the tubes, angular, compound. Stipe 4-7 cm. long, 6-12 mm. thick, whitish to yellowish or brownish at the base, punctate with dark-brown elements, subequal, stuffed then hollow, slightly reticulate at the apex; ring whitish, thin-membranous. Flesh yellowish, unchanging.

Spores olivaceous under the microscope, elliptical, $8-11 \times 3.5-4.5 \mu$ ($8.5 \times 3.5 \mu$).

Aug. 11, 1942, W. F. Chiu, Shishan, Kunming (7849 and 7858). Under *Pinus Armandii*.

The present fungus agrees closely with the European form except that the dotted elements on the stipe may extend downward below the ring.

BOLETUS LUTEUS Fr. ex L.

Pileus 3.5-5 cm. in diameter, convex to plano-convex, "Cinnamon Buff" to "Tawny," viscid when young and moist, becoming glabrous and shining when dry, finely virgate with age. Tubes 3-4 mm. long, yellow, becoming olive, short-decurrent; pores minute, 2-3 per mm., yellow. Stipe 2.5-4 cm. long, 5-10 mm. thick, yellowish, reticulate on the upper part of the stem from the decurrent walls of the pores, punctate with brownish elements above

and below the ring; ring thin-membranous, brownish and tinged with purplish; veil becoming shredded but sheathing the lower part of the stipe when young. Flesh firm, whitish or yellowish.

Spores olivaceous under the microscope, elliptical, spindle-elliptical, $8-9 \times 3-3.5 \mu$ ($8 \times 3 \mu$).

Aug. 24, 1940, F. L. Tai, Kunming (5573); Aug. 5, 1942; Tapugi, W. F. Chiu (7856).

The spores of the Yunnan form are very close to those described by Singer (Florida Boletineae II: 271, 1945).

BOLETUS THIBETANUS Pat.

Pileus 2-3.5 cm. in diameter, pulvinate to convex, "Liver Brown," pale toward the margin, reticulate in the central portion, very viscid when moist, densely covered with dark-brown-dotted elements when young; margin often appendiculate with gelatinous veils. Tubes 4-9 mm. long, "Lemon Chrome" or "Chalcedony Yellow," adnate-uncinate; pores up to 1 mm. across, concolorous with the tubes, angular, simple. Stipe 3-6 cm. long, 3-6 mm. thick, "Vinaceous Pink," paler near the apex, sometimes "Vinaceous Tawny," glabrous or somewhat fibrillose, viscid when young or when moist, slender, sometimes tapering downward. Flesh white, turning slightly pinkish.

Spores olivaceous under the microscope, elliptical-fusiform, $8-10-12 \times 4-5 \mu$ ($10 \times 4 \mu$).

Aug. 6, 1938, C. C. Cheo, Haiyuantze, Kunming (8042); July 22, 1942, W. F. Chiu, Shishan, Kunming (7896); Aug. 11, 1942, the same place (7851); June 1938, W. F. Chiu, Mt. Tsing Cheng, Szechwan (Chiu 304).

Patouillard in his original diagnosis of this species did not mention the gelatinous membrane and the dotted elements on the cap. Since the species is very common in Yunnan, individuals in different stages of development have been studied. It is found that the plant is covered with a gelatinous veil when young, and with minute granules scattering among the veins on the cap. The reticulation is the result of the shrinking of the gelatinous membrane. Thus the veins on the cap become distinct only when the gelatinous membrane is dried and shrunken. The granules or dotted elements which are very distinct when young can be entirely lost when old. Patouillard's description seems to be based only on a certain stage of development.

BOLETUS PLACIDUS Bon.

Pileus 2-11 cm. in diameter, convex to plane, white, "Buff Yellow" to "Amber Brown," very viscid when moist, shining when dry. Tubes 4-6 mm. long, "Sulphur Yellow," decurrent; pores 1-2.5 mm. across, concolorous with the tubes, usually dotted with cremeous-yellow glandules which become dark brown with age, usually angular, sinuous. Stipe 3-5 cm. long, 10-13 mm. thick, whitish to yellowish, dotted with yellowish glandules which turn brown to black with age, subequal or slightly tapering downward, central or excentric, stuffed. Flesh solid, yellowish to yellow, sometimes pinkish at the base of the stipe.

Spores olivaceous under the microscope, ellipsoid, $6-9(10) \times 3-4 \mu$ ($8 \times 3 \mu$).

July 23, 1938, C. C. Cheo, Shishan, Kunming (7902); Aug. 1938, Chunghotze, Tali, C. C. Cheo (7775); Sept. 1938, C. C. Cheo, Chichushan (7777 and 7780); Aug. 24, 1940, F. L. Tai, Tapugi, Kunming (5546); Dec. 1941, W. F. Chiu et C. I. Wu, Tiehfungan, Kunming (7714 and 7739); June 30, 1941, C. C. Cheo, Miaokaotze, Kunming (7705); July 8, 1942, W. F. Chiu, Tapugi, Kunming (7854, 7870 and 7874). Under *Pinus yunnanensis*.

Kallenbach thoroughly studied this species and gave excellent paintings (Die Pilze Mitteleuropas B.I.S.: 101, 1923-34). According to the opinion of Kallenbach, Bonordon's species should not be placed in the genus *Gyrodon*. The color plate given by Bresadola (Icon. Myc. T. 944) is rather poor. The writer has collected this plant under pines for several years, and has been able to study its various stages of development. The Yunnan plant is exactly identical with the European form. However, some young fruiting bodies, as far as observed in the Yunnan collections, do appear to be of the *Gyrodon* type, i.e., with very short and decurrent tubes. The color of the cap varies from white, "Buff Yellow" to "Amber Brown," whereas that of the granules on the stipe is cream-yellow when young, and always becomes black with age. The length of time that a sporophore requires to take for passing from one stage of growth to the other is variable. The granules on the stipes of some specimens may remain cremeous yellow for a very long period, whereas in other cases, such granules soon darken.

BOLETUS GRANULATUS Fr. ex L.

Pileus 2.4–4.5 cm. in diameter, convex to plano-convex, glabrous, viscid, "Vinaceous Pink" to "Chocolate Brown." Tubes 2–3 mm. long, yellow, short, subdecurrent or adnate; pores less than 1 mm. across, concolorous with the tubes, dotted with creamy granules, simple, angular. Stipe 3–7 cm. long, 6–12 mm. thick, whitish, dotted with dark brown or reddish-brown granules, subequal. Flesh whitish to yellowish in the stipe, unchanging.

Spores olivaceous under the microscope, spindle-shaped or fusiform, $6-9 \times 2-3 \mu$ ($6 \times 2 \mu$).

Aug. 28, 1938, Chunghotze, Tali, C. C. Cheo (7802).

BOLETUS PORPHYROSPORUS Fr.

Pileus 5 cm. in diameter, convex, "Mummy Brown," to "Bister," velvety, rugose; margin slightly incurved or straight. Tubes 3–8 mm. long, "Vandyke Brown," round and free; pores 5–7 mm. across, concolorous with the tubes, angular to roundish. Stipe 11 cm. long, 7 mm. thick, brownish, punctate and streaked with dark ("Vandyke Brown") elements, rather slender. Flesh whitish, becoming pinkish in the stipe, fibrous.

Spores ochraceous or brown under the microscope, elliptical-fusiform, $14-17 \times 6-7 \mu$ ($16 \times 6 \mu$).

Aug. 28, 1938, S. T. Chao, Shishan, Kunming (8038).

This is a slender-stiped form.

Boletus virens sp. nov.

Pileo 2.5–8 cm. lato, hemispherico dein convexo, mox prope plano, sicco, glabro, interdum minutissime pruinoso, primitus "Danube Green," "Saccardo Olive," aetate "Primuline Yellow" v. "Mustard Yellow," saepe olivaceo-squamuloso et rimoseo-areolato; tubulis usque ad 20 mm. longis, albidis dein "Light Congo pink," ventricosis v. posteriore angulatis; poris 1–2 mm. latis, aetate majusculis, usque ad 4 mm. latis, "Congo Pink," rotundatis; stipite 2–7 cm. longo, 7–20 mm. crasso, subaequali v. subventricoso, "Pale Chalcidony Yellow," "Pinard Yellow," obscure reticulato v. lateritio-striato v. puniceo-punctato, basi saepe "Salmon Orange" v. "Orange Chrome"; carne flava, immutabili; sporis hyalinis, ellipsoideis, $9-11-14 \times 5-6 \mu$ ($12 \times 6 \mu$).

Pileus 2.5–8 cm. in diameter, convex to nearly plane, "Danube Green," "Kronberg Green" or "Saccardo's Olive" when young, "Primuline Yellow" to "Mustard Yellow" when old, usually covered with "Olive-Lake" squamules which are not distinct until the pellicle is areolately cracked with age. Tubes up to 2 cm. long,

light "Congo Pink," ventricose to angular posteriorly, free from or depressed around the stipe; pores large, about 1–2 mm. across (4 mm. across when old), concolorous with the tubes, roundish. Stipe 2–7 cm. long, 7–20 mm. thick, "Pale Chalcedony Yellow" or "Pinard Yellow," indistinctly reticulate with "Olive Lake" veins, sometimes tinged reddish in certain portions, sometimes tinged "Empire Yellow" at the basal portion, stuffed. Flesh pale "Pinard Yellow," unchanging.

Spores pale olivaceous under the microscope, elliptical, $11\text{--}14 \times 5.5\text{--}6 \mu$ ($12 \times 6 \mu$).

July 23, 1938, Chenkung, C. C. Cheo (7882); July 22, 1942, W. F. Chiu, Shishan, Kunming (7877); Aug. 7, 1942, W. F. Chiu, Miaokaotze, Kunming (7859), **Type**. Usually under *Keteleeria Evelyniana*.

The color of the cap varies much according to the stage of development. Young fruiting bodies with green cap and the old ones usually with yellow to olive cap might be mistaken for two distinct species if the variations were not studied in the field. This species is close to *B. felleus*, but the cap of the latter is never green, so far as the available records show.

***Boletus punctato-fumosus* sp. nov.**

Pileo 2–3.5 cm. lato, hemispherico v. pulvinato dein convexo, "Snuff Brown," minute pubescenti; tubulis 7 mm. longis, carneis, ad stipitem depressis; poris angulatis, 1 mm. latis, carneis; stipite 5–6 cm. longo, 7–8 mm. crasso, subaequali, flavo, rufo-brunneo punctato; carne flava; sporis pallidiore olivaceis, ellipsoideis, $9\text{--}12 \times 5\text{--}6 \mu$ ($11 \times 5 \mu$).

Pileus 2–3.5 cm. in diameter, hemispherical to pulvinate, becoming flesh-colored, depressed around the stipe; pores less than 1 mm. across, concolorous with the tubes, simple, angular. Stipe 5–6 cm. long, 7–8 mm. thick, "Empire Yellow," punctate with dark-brown elements, subequal, slightly tapering upward. Flesh whitish, yellowish at the base of the stipe, unchanging.

Spores pale olivaceous under the microscope, ellipsoid, $9\text{--}11 \times 5\text{--}6 \mu$ ($11 \times 5 \mu$).

Sept. 14, 1938, C. C. Cheo, Chichushan, Binchwan (7803), **Type**. Under *Pinus Armandii*.

In the *Tylopilus* group of the boletes, three species of Asia, namely *B. punctato-fumosus*, *B. javanicus*, and *B. roseolus*, are closely related. The first one can be easily distinguished from the

latter two by the dark "Snuff Brown" cap and the yellow, punctate stipe.

Boletus roseolus sp. nov.

Pileo 2-3 cm. lato, convexo v. plano-convexo, pubescenti, "Carnelian Red" v. "Pale Brazil Red," interdum pallescenti; tubulis 3-5 mm. crasso, apice flavido, basi "Empire Yellow" sed sursum e medius "La France Pink," pubescenti; carne flavida; sporis pallidiore olivaceis, $9-14 \times 5-6 \mu$ ($11 \times 5 \mu$).

Pileus 2-3 cm. in diameter, convex to plano-convex, pubescent, "Carnelian Red" to "Pale Brazil Red," occasionally becoming much paler. Tubes 3-5 mm. long, flesh-colored; pores 1 mm. across, concolorous with the tubes. Stipe 4.5-7 cm. long, 5-10 mm. thick, yellowish at the apex, "Empire Yellow" at the base, sometimes "La France Pink" from the base to the middle, pubescent. Flesh yellowish.

Spores pale olivaceous under the microscope, elliptical, $9-14 \times 5-6 \mu$ ($11 \times 5 \mu$).

Sept. 7, 1938, H. S. Yao, Chichushan, Binchwan (7807), Type. Under *Pinus Armandii*; July to Aug. 1938, C. C. Cheo, Kunming (7891). Under *Pinus yunnanensis*.

This species is close to the Java plant, *B. javanicus* P. Henn., but is distinct from the latter in the pubescent cap, the yellow and pink stipe and the broader spores.

BOLETUS VELATUS (Rostr.) Sacc.

Pileus 1-3 cm. in diameter, convex, dry, "Auburn" to "Chestnut Brown," rimoso-squamose; margin stellately appendiculate with the extruding edge of the membrane of the cap (or the extruding part wrapping around the stipe). Tubes 5 mm. long, incarnate, free; pores 0.5-1 mm. across, whitish, angular, simple. Stipe 4-8 cm. long, 5-6 mm. thick, "Ochraceous Salmon" above, "Tawny" below, glabrous, equal, stuffed. Flesh white, usually pinkish beneath the pellicle.

Spores elliptical to subfusiform, pale olivaceous under the microscope, $12-16 \times 4-6 \mu$ ($14-16 \times 5 \mu$).

Aug. 7, 1942, Miaokaotze, Kunming, W. F. Chiu (8047); Sept. 4, 1942, the same locality, S. C. Shen (7850). Under *Keteleeria Evelyniana*.

The spore measurements of this species are lacking in the original diagnosis. Externally the Yunnan plant appears to be identical with the Siamese plant.

Boletus albofarinaceus sp. nov.

Pileo 5 cm. lato, convexo, sicco, albo, albofarinaceo; tubulis 3 mm. longis, "Pinkish Vinaceous" liberis; poris 7-10 mm. latis, "Dark Vinaceous"; stipite 6-7 cm. longo, 7-8 mm. crasso, aequali v. deorsum ventriculoso, albo, brunneo-striato, basi albido; carne alba, basi flavida, immutabili; sporis hyalinis, ellipsoideis, $11-14 \times 5-7 \mu$ ($12 \times 6 \mu$).

Pileus 5 cm. in diameter, convex, dry, white, white-pulverulent. Tubes 3 mm. long, "Pinkish Vinaceous," free from the stipe, ventriculose; pores 0.7-1 mm. across, "Dark Vinaceous," simple, angular. Stipe 6-7 cm. long, 7-8 mm. thick, white, yellowish at the base, pulverulent, striate with brown fibrils, slightly dilated toward the base. Flesh whitish, fibrous, yellowish at the basal part of the stipe, unchanging.

Spores hyaline under the microscope, ellipsoid, $11-14 \times 5-7 \mu$ ($12 \times 6 \mu$).

Aug. 28, 1938, Kunming, F. L. Tai (8037), **Type**.

This plant is obviously of the *Tylopilus* type. However among boletes of this group, species with white and white-pulverulent caps have not been recorded.

BOLETUS UMBRINUS Pers.

Pileus 2-8 cm. in diameter, pulvinate, becoming plano-convex, "Russet" to "Pecan Brown" or "Vandyke Brown," subglabrous, rimoso-areolate with age; margin wavy. Tubes 5-8 mm. long, whitish, adnate to subdecurrent; pores 5-8 cm. across, whitish becoming brown, angular, simple. Stipe 3.5-9 cm. long, 1-4 cm. thick, "Pecan Brown" with dark-brown-reticulated veins at the apex, ventricose when old, thick. Flesh solid, whitish, slightly pinkish when exposed to the air.

Spores olivaceous under the microscope, elliptical to subfusiform, $8-12 \times 3-5 \mu$ ($11 \times 4 \mu$).

Aug. 11, 1941, Tapugi, Kunming, C. C. Cheo (7694); Aug. 20, 1942, the same locality, W. F. Chiu (7857); July 22, 1942, Shishan, Kunming, W. F. Chiu (7869).

According to the original diagnosis, *B. umbrinellus* Pat. et Baker does not seem to differ from *B. umbrinus* except that the spores are narrower. The width of the spores of the Yunnan plant lies between these two species, i.e., $8-12 \times 3-5 \mu$. The writer is of the opinion that *B. umbrinellus* might be synonymous with *B. umbrinus*.

BOLETUS CROCIPODIUS Letel.

Pileus 7-14 cm. in diameter, hemispherical, becoming convex then plane and reflexed, somewhat viscid when moist, "Mars Yellow," tomentose, rugose when young, rimoso-areolate with age; margin extended beyond the tubes forming a narrow collar closely applied to the stipe when young. Tubes 8-23 mm. long, "Empire Yellow," becoming "Citron Green" with age, usually free; pores minute, less than 1 mm. across, concolorous with the tubes. Stipe 5-12 cm. long, 2.5-4.5 cm. thick, "Antimony Yellow," punctate with concolorous elements, equal or slightly tapering upward, rarely tapering downward, stuffed. Flesh solid, white, changing to lilac in the stipe, yellowish above the tubes when exposed to the air.

Spores olivaceous under the microscope, elliptical to ellipsoid-oblong, $9-12-(14) \times 3-5 \mu$ ($11 \times 4 \mu$).

July 1941, Tapugi, F. L. Tai et C. C. Cheo (7883, 7749, 7755, 7741 and 7738); July 11, 1942, Tapugi, Kunming, Mrs. Chiu (7829).

The present fungus is very close to *B. rimosus* (*B. tessellatus* etc.) except that it has smaller spores. It is also distinct from *B. fuscescens* Clal., which may be synonymous with *B. crocipodius*, in the different color and the non-bulbous stipe.

Gilbert maintains that *Krombholzia crocipodia* (Letel.) Gilb. should be the right name, and points out at the same time that *B. rimosus* Vent. is distinct from the former (Les Bolets: 179, 1931). Singer, however, considers *K. luteopora* (Bouchinot) as the right name with *K. crocipodia* as a synonym. His reason for the change is that before the publication of *B. luteoporus*, there was no valuable diagnosis of *B. crocipodius* (Rev. Myc. 3: 188, 1938). In Singer's recent publication, *K. luteopora* has been made a synonym of *Leccinum nigrescens* (The Boletineae of Florida III: 117, 1947). In spite of all such changes, the writer is using the present name, because it has been generally accepted.

Boletus violaceo-fuscus sp. nov.

Pileo 4-7 cm. lato, semigloboso, dein convexo, prope plano, "Blue Violet" v. "Maroon Purple," mox pallenscenti, raro rugoso, minutissime velutino; tubulis 3-6 mm. longis, albis dein flavidis, sinuatis v. liberis; poris minutis, 1-2 in uno mm. latis, albis, aetate flavidis; stipite 5-7 cm. longo, 1-2 cm. crasso, subaequali, basi ventricosus, pilei concolore, reticulato; carne alba, immutabili; sporis olivaceis, fusoido-ellipticis, $12-14 \times 5-6 \mu$ ($12 \times 6 \mu$).

Pileus 4–7 cm. in diameter, hemispherical, becoming plano-convex, dark purple to dark "Blue Violet," sometimes fading to dark "Maroon Purple," usually smooth, rarely rugose, minutely velvety. Tubes 3–6 mm. long, at first white, becoming yellowish, sinuate or free, depressed around the stipe; pores minute, 0.5–1 mm. across, white becoming yellowish with age or when bruised. Stipe 5–7 cm. long, 1–2 cm. thick, subequal or ventricose at the basal portion, concolorous with the cap but often paler, distinctly reticulate with white veins. Flesh white, solid, becoming spongy when old or when tunneled by worms.

Spores olivaceous under the microscope, fusiform-elliptical, $12-14 \times 5-6 \mu$ ($12 \times 6 \mu$).

Sept. 2, 1938, S. T. Chao, Kunming market (7007, **Type**, and 7008); July 8, 1938, Chengtu market, W. F. Chiu (Chiu 74); Aug. 1942, the writer encountered this plant in *Quercus* woods (*Q. variabilis*). The size of cap reaches 25 cm. in diameter, and the stipe is as thick as 4 cm. Unfortunately this specimen had collapsed before it could be delivered to the laboratory for preservation.

This plant is distinct among the white-tubed boletes for its dark violet color and the beautiful white reticulation on the stipe. Specimens were first collected from the Chengtu market where it is known as edible. Studies in detail and descriptions were not made until it was encountered again in the Kunming market. Its association with *Quercus variabilis* was recognized in a field near Tapugi, Kunming.

BOLETUS AEREUS Fr. ex Bull.

Pileus 3–11 cm. in diameter, hemispherical, becoming convex and plano-convex, dry, minutely velvety or becoming glabrous, dark "Chestnut Brown," "Natal Brown" to "Drab," nearly "Pale Pinkish Buff" in some cases; margin sometimes wavy. Tubes 4–8 mm. long, white becoming "Vinaceous Buff," free or uncinat; pores 0.5–1 mm. across, grayish-white, simple, round, uneven. Stipe 3–9 cm. long, 1.5–5 cm. thick, "Medal Bronze," "Clay Color" or "Mikado Brown," paler in the upper part, distinctly reticulate with dark-brown veins, ventricose when young, subequal when old, central or excentric. Flesh firm, white, sometimes turning pinkish when exposed to the air or becoming yellow where bruised or tunneled by worms, rarely changing to bluish at the base of the stipe.

Spores olive under the microscope, oblong-fusiform or oblong-elliptical, $9-12 \times 4-5 \mu$ ($11 \times 4 \mu$).

July 13, 1938, Kunming market, C. C. Cheo et S. T. Chao (5481, 5568 and 7898); July to Aug. 1941, Tapugi, Kunming, F. L. Tai et C. C. Cheo (7747, 7754, 7742, 7463, 7894 and 7723). Usually under *Quercus variabilis*.

The variation of the cap color has been carefully observed in the Yunnan plant. The color ranges from dark "Chestnut Brown," "Natal Brown," "Drab" to nearly "Pale Pinkish Buff." The white tubes do not change to yellowish or yellowish-green but to "Vinaceous Buff." The stipe color varies from "Medal Bronze," "Clay Color" to "Mikado Brown."

BOLETUS RAVENELII Berk. et Curtis

Pileus 3-6 cm. in diameter, hemispherical to convex, then slightly depressed, yellow, sometimes tinged pinkish or reddish, generally yellow-pulverulent; margin appendiculate with yellow membrane. Tubes 6-9 mm. long, yellow, becoming ochraceous; pores more or less 1 mm. across, "Aniline Yellow" becoming ochraceous, angular. Stipe 3-9 cm. long, 0.5-1 cm. thick, concolorous with the cap but paler above the ring, farinaceous, equal, stuffed, sometimes tinged reddish. Flesh whitish, turning bluish when exposed to the air.

Spores pale "Lumiere Green" under the microscope, ellipsoid, $8-11(12) \times 4.5-5.5 \mu$ ($9 \times 4.5 \mu$).

Sept. 4, 1938, Kunming market, F. L. Tai (7701); July 28, 1938, Mt. Omei, Szechwan (7688). Under coniferous tree.

Boletus pseudostrobilomyces sp. nov.

Pileo 6.5 cm. lato, semigloboso, sicco, "Vinaceous Cinnamon" v. "Cinnamon," strobilaceo-squarroso, "Verona Brown"-squamoso; tubulis 15 mm. longis, "Lemon Yellow," adnatis; poris mediis, concolore, rotundatis; stipite 14 cm. longo, 8 mm. crasso, "Cinnamon," "Russet," fibrilloso, aequali, farcto; carne alba, caerulescente; velo griseo, ad marginem appendiculato; sporis ochraceis, ellipsoideis, $15-19 \times 7.5-8 \mu$.

Pileus 6.5 cm. in diameter, hemispherical, dry, "Vinaceous Cinnamon," covered with thick, "Verona Brown," strobilaceous scales. Tubes 15 mm. long, "Lemon Yellow," adnate; pores moderately large, concolorous with the tubes, roundish. Stipe 14 cm. long,

8 mm. thick, "Cinnamon," minutely striate with "Russet" fibrils, equal, pithed. Flesh white changing immediately to blue when exposed to the air. Veil grayish, membranous, persistent and appendiculate to the margin of the cap.

Spores smooth, brown or dark ochraceous under the microscope, elliptical, $15-19 \times 7.5-8 \mu$.

Aug. 2, 1938, W. F. Chiu, Mt. Omei, Szechwan (Chiu 121), **Type.** Under *Machilus bournei*.

The pyramidal scales on the cap of this fungus are somewhat like those of *Strobilomyces floccopus*, but according to the spore-form and other macroscopical characters, it belongs evidently to the *Xerocomus* group. The Australian species *S. velutipes* has been described as having smooth or partially smooth spores. However, the present plant differs from the Australian species in the entirely different colors, the non-blackening of the flesh, and the smooth, elliptical and longer spores.

***Boletus subpaludosus* sp. nov.**

Pileo 3.5-4 cm. lato, convexo, "Pale Vinaceous Tawny," glabro; tubulis 4-5 mm. longis, flavidis v. flavis, caerulescentibus, adnatis v. breviter decurrentibus; poris flavis, 0.8-1 mm. latis, angulatis; stipite 4-8 cm. longo, 4-6 mm. crasso, subaequali, "Light Vinaceous Cinnamon" v. "Cinnamon," glabro, saepe striato v. leniter flexuoso; carne flava, caerulescente; sporis fuscolivaceis, ellipsoideis, $8-12 \times 4-5 \mu$ ($11 \times 4.5 \mu$).

Pileus 3.5-4 cm. in diameter, convex, "Pale Vinaceous Tawny," glabrous. Tubes 4-5 mm. long, yellow, changing to blue when bruised, sinuate, decurrent with a short tooth; pores concolorous with the tubes, 0.8-1 mm. across, angular to somewhat labyrinthiform. Stipe 4-8 cm. long, 4-6 mm. thick, subequal or slightly tapering near the base, "Light Vinaceous Cinnamon" to "Cinnamon," glabrous, usually striate, slightly curved. Flesh yellow, changing to blue when exposed to the air.

Spores dark olive under the microscope, elliptical or ellipsoidal, $8-12 \times 4-5 \mu$ ($11 \times 4.5 \mu$).

Aug. 1940, Tapugi, Kunming, F. L. Tai (8033), **Type.**

This plant appears macroscopically to be close to *B. paludosus*, but differs from the latter in that its flesh and tubes change to blue when bruised. It also differs from *B. yunnanensis* in the changing flesh and the glabrous cap.

BOLETUS COMMUNIS Bull. var. *MUTATIS* Schultz

Pileus 3–3.5 cm. in diameter, convex to nearly plane, "Buffy Brown," "Yellow Olive," with minute tomentum which is tinged "Saccardo Olive," usually finely areolate, reddish at the wounded spot on the cap. Tubes "Amber Yellow," ventricose, up to 6 mm. long, free or uncinat, deeply depressed around the stipe with age, slowly changing to green when bruised; pores around 1 mm. across, concolorous with the tubes. Stipe 3–3.5 cm. long, 4–5 mm. thick, slightly tapering downward, yellow at the apex, "Argus Brown" below, striate with reddish fibrils. Flesh white, reddish beneath the cuticle, yellowish in the middle, slowly changing to blue in the cap and to reddish in the stipe when exposed to the air.

Spores olivaceous under the microscope, elliptical, $9-11 \times 4-5 \mu$ ($11 \times 4.5 \mu$).

June 20, 1943, S. Y. Yin, Chiunchutze, Kunming (8200). In deciduous woods on damp slope.

The typical form of this species has not been collected in this province. *B. chrysenteron* Bull., which is a synonym of *B. communis*, has been recorded by Teng (S. C.) from Kiangsu.

BOLETUS TOMENTIPES Earle

Pileus 3.5–11 cm. in diameter, convex to plane, "Brussels Brown," minutely tomentose, becoming subglabrous, sometimes rimose-areolate. Tubes about 1 cm. long, "Antimony Yellow," free to uncinat, depressed around the stipe; pores minute, less than 1 mm. across, concolorous with the tubes, somewhat angular. Stipe 5–8 cm. long, 15–20 mm. thick, "Light Coral Red" at the apex, "Brussels Brown" downward, minutely tomentose, subequal, stuffed. Flesh whitish or brownish, changing to blue when bruised.

Spores dark olive under the microscope, elliptical, $9-12 \times 5-6 \mu$ ($11 \times 5.5 \mu$).

Sept. 13, 1938, Chichushan, Binchwan, C. C. Cheo (7804).

This form accords closely with the original description of *B. tomentipes* except that the tubes do not turn red when bruised.

Boletus nigropunctatus sp. nov.

Pileo 6–7 cm. lato, hemisphaerico, "Saccardo's Umber," fusco-brunneo-v. "Sepia"-punctato, tubulis usque ad 12 mm. longis, flavis dein ochraceis, ad stipitem depressis; poris majusculis, hexagonalibus, flavis dein ochraceis;

stipite 6-7 cm. longo, 5-8 mm. crasso, pileo subconcolore; saepe pallidiore, glabro, aequali; carne flavida, caerulescente; sporis ochraceis, ellipsoideis, $6-8 \times 3-4 \mu$.

Pileus 6-7 cm. in diameter, hemispherical, "Saccardo's umber," punctate with dark brown or "Sepia" elements, dry. Tubes 12 mm. long, yellowish, becoming ochraceous with age, convex, depressed around the stipe; pores large, hexagonal, concolorous with the tubes. Stipe 6-7 cm. long, 5-8 mm. thick, subconcolorous with the cap, usually paler, equal, stuffed. Flesh yellowish, changing to blue when exposed to the air.

Spores ochraceous under the microscope, ellipsoidal, $6-8 \times 3-4 \mu$.

Aug. 4, 1938, Mt. Omei, W. F. Chiu (Chiu 262), **Type**. In coniferous woods.

Boletus instabilis sp. nov.

Pileo 6-7 cm. lato, pulvinato, obliquo, "Ochraceous Tawny," subglabro, tessellato-areolato; tubulis usque ad 10 mm. longis, flavis, rotundatis; stipite 6-7 cm. longo, 1.5-2.5 cm. crasso, "Pinkish Cinnamon," glabro, deorsum attenuato, crasso, excentrico; carne alba, fracta pilei punicea, stipiti brunneo-striata; sporis olivaceis, ellipsoideis v. subfusiformibus, $9-14 \times 4.5 \mu$ ($11 \times 4.5 \mu$).

Pileus 6-7 cm. in diameter, pulvinate, sloping, "Ochraceous Tawny," subglabrous, tessellately areolate; margin notched at a point. Tubes up to 10 mm. long, yellow, changing to blue when bruised, somewhat short-decurrent; pores 0.5-1 mm. across, concolorous with the tubes, roundish. Stipe 6-7 cm. long, 1.5-2.5 cm. thick, extremely excentric to nearly lateral. Flesh white, pinkish in the cap and streaked with brown fibers in the stipe when cut.

Spores olive under the microscope, elliptical to subfusiform, $9-14 \times 4-5 \mu$ ($11 \times 4.5 \mu$).

July 23, 1938, C. C. Cheo, Chenkung (7895), **Type**.

The present fungus differs from *B. paludosus* Mass. in the nearly lateral stipe and the always tessellately areolate cap.

Boletus Cheoi sp. nov.

Pileo 1.5-5 cm. lato, pulvinato dein convexo, saepe subumbonato, sicco, primitus "Liver Brown," aetate "Cinnamon Rufous," fusco-brunneo-fibrilloso e squamuloso; tubulis 4-12 mm. longis, "Amber Yellow" v. "Citron Yellow," flavidis, caerulescentibus, adnatis; poris 1-2 mm. latis, flavis; stipite 3-6 cm. longo, 1-7 mm. crasso, cylindrico, saepe sursum attenuato, "Pinkish Buff" v. "Tawny Olive," apice pallidiore caerulescente; sporis olivaceis, ellipsoideis, $8-11 \times 4-5 \mu$ ($9 \times 5 \mu$).

Pileus 1.5–5 cm. in diameter, pulvinate then convex, subumbonate, dry "Liver Brown" when young, "Cinnamon Rufous" with age, covered with dark brown fibrillose scales. Tubes 4–12 mm. long, "Amber Yellow" or "Citron Yellow," turning blue when bruised, adnate; pores 1–2 mm. across, concolorous with the tubes. Stipe 3–6 cm. long, 1–7 mm. thick, slender, usually tapering upward, "Pinkish Buff" or "Tawny Olive" at the top, glabrous, stuffed. Flesh whitish turning brownish or reddish when exposed to the air.

Spores olivaceous under the microscope, elliptical, $8-11 \times 4-5 \mu$ ($9 \times 5 \mu$).

Sept. 11, 1938, Chichushan, Binchwan, C. C. Cheo (7692), **Type**; Aug. 24, 1938, Tali, C. C. Cheo (7700); July 19, 1938, Shishan, Kunming, T. K. Yien (7698 and 7699); Aug. 1938, the same locality, F. L. Tai et C. C. Cheo (7697 and 7715); July 12, 1943, Shishan, Kunming, W. F. Chiu (8025). Usually in woods of *Keteleeria Evelyniana*.

In Yunnan there are two small boletes of the *Xerocomus* type: *B. Cheoi* and *B. punctilifer*. They are both close to *B. decorus*, but can be readily distinguished from the latter by the yellow and pinkish flesh, and the shorter spores which never reach 13μ long. The present species can also be separated from *B. punctilifer* by the umbonate cap, the broad adnate tubes and the glabrous stipe.

***Boletus punctilifer* sp. nov.**

Pileo 3–8 cm. lato, convexo dein plano, sicco, punctato-tomentoso, primitus "Argus Brown," "Liver Brown" dein "Hay's Russet" v. "Cinnamon Rufous"; tubulis 10 mm. longis, "Amber Yellow" v. "Citron Yellow," sinuatis; poris circa 1 mm. latis, angulatis, leviter puniceis dein flavis, aetate cyanescentibus; stipite 4–7 cm. longo, 7–10 mm. crasso, subaequali v. subventricoso, supra "Pinkish Cinnamon," infra flavido, minute punctato e fibrilloso, glabrescenti, farcto; carne pilei flavida, stipiti punicea; sporis breviter ellipsoideis, olivaceis, $7-10 \times 4.5-5.5 \mu$ ($9 \times 4.5 \mu$).

Pileus 3–8 cm. in diameter, convex becoming plane, punctate with small tufts of tomentum, "Argus Brown" or "Liver Brown," becoming "Hay's Russet" to "Cinnamon Rufous," dry. Tubes 10 mm. long, "Amber Yellow" to "Citron Yellow," sinuate; pores around 1 mm. across, angular, slightly pinkish then yellowish, turning blue with age. Stipe 4–7 cm. long, 7–10 mm. thick, subequal to ventricose, "Pinkish Cinnamon" in the upper part, yellow below, finely punctate and fibrillose, becoming glabrous when old, solid. Flesh yellowish in the cap, pinkish in the stipe.

Spores short ellipsoid, olivaceous under the microscope, $7-10 \times 4.5-5.5 \mu$ ($9 \times 4.5 \mu$).

June 21, 1941, Miaokaotze, Kunming, C. C. Cheo (7735); Aug. 1938, Shishan, Kunming, W. F. Chiu (7748); July 8, 1942, Tapugi, Kunming, W. F. Chiu (7860, **Type**, and 7873).

This plant is closely related to *B. Cheoi* except that the convex pileus is never umbonate, and the punctate stipe never smooth when young. Another character which serves to separate these two species is the attachment of the tubes. In the present fungus, the tubes are always free from the stipe, while in *B. Cheoi* they are broadly adnate.

Boletus puniceus sp. nov.

Pileo 5 cm. lato, plano, "Old Rose," minute tomentoso; tubulis usque ad 17 mm. longis, "Pinard Yellow," immutabilibus, sinuatis; poris majusculis, 2-2.5 mm. latis, flavis, rotundatis e angulatis; stipite 11 cm. longo, 8-12 mm. crasso, leviter sursum attenuato, pileo concolore, dense floccoso e pubescenti; carne alba, immutabili; sporis olivaceis, ellipticis, $12-19 \times 7-8 \mu$ ($16 \times 8 \mu$).

Pileus 5 cm. in diameter, plane, "Old Rose," minutely tomentose. Tubes up to 17 mm. long, "Pinard Yellow," unchanging, sinuate; pores large, 2-2.5 mm. across, concolorous with the tubes, roundish to angular. Stipe 11 cm. long, 8-12 mm. thick, concolorous with the cap, densely floccose and pubescent, slightly tapering upward. Flesh white, yellowish at the base of the tube layer, unchanging.

Spores olivaceous under the microscope, elliptical, large, $12-19 \times 7-8 \mu$ ($16 \times 8 \mu$).

Sept. 30, 1942, Miaokaotze, Kunming, H. R. Wang et K. S. Wu (7825), **Type**.

The distinguishing characters of the species are the large spores which are rarely found in species of the *Xerocomus* group, and the large angular pores which are also rare in the related species.

Boletus yunnanensis sp. nov.

Pileo 2.2-3.8 cm. lato, convexo, "Argus Brown," velutino; tubulis "Lemon Yellow" dein "Aniline Yellow," 4-5 mm. longis, breviter decurrentibus; poris 0.7-1 mm. latis, aetate ochraceis, angulatis; stipite 3-5 cm. longo, 3-10 mm. crasso, aequali v. leniter sursum attenuato, basi leviter bulboso, "Avellaneous," glabro; carne pilei flava, stipiti alba; sporis olivaceis, ellipsoideis v. subellipticis, $7.5-11 \times 3-4.5 \mu$ ($9 \times 4.5 \mu$).

Pileus 2.2–3.8 cm. in diameter, convex, "Argus Brown, distinctly velvety. Tubes "Lemon Yellow," becoming "Aniline Yellow," 4–5 cm. long, decurrent with a short tooth; pores 0.7–1 mm. across, concolorous with the tubes, turning ochraceous with age, angular, somewhat irregular. Stipe 3–5 cm. long, 3–10 mm. thick, equal or slightly tapering upward, slightly bulbous at the base. Flesh yellow in the cap, white in the stipe.

Spores slightly olivaceous under the microscope, elliptical to sub-elliptical, $7.5\text{--}11 \times 3\text{--}4.5 \mu$ ($9 \times 4.5 \mu$).

July, 1938, Shishan, Kunming, F. L. Tai (7900), **Type**.

This species is usually confused with *B. subpaludosus* especially when dry, but the velvety cap of the former can still be recognized even when dried in contrast with the glabrous cap of *B. subpaludosus*. The unchanging flesh and the short spores of the present species also serve to separate it from *B. paludosus* Mass.

BOLETUS SYLVESTRIS ? Petch

Pileus 6 cm. in diameter, rarely plane, "Vinaceous Rufous," "Bay" at the center, minutely tomentose, becoming glabrous. Tubes 6 mm. long, yellow, adnate-uncinate; pores about 1 mm. across, concolorous with the tubes, angular, simple. Stipe 3.5 cm. long, 1 cm. thick, "Tawny," tomentose, paler toward the base, slightly ventricose. Flesh white, turning slightly pinkish.

Spores olivaceous under the microscope, elliptical, $7.5\text{--}11 \times 4.5\text{--}6.5 \mu$ ($9 \times 5 \mu$).

July 31, 1938, Chiunchutze, Kunming, C. C. Cheo (8036).

This plant is externally close to *B. sylvestris* Petch, but as the shape and size of the spores of the latter were not given by Petch in his original diagnosis, the writer cannot be certain that the Ceylon plant is exactly identical with ours. The spores of the present plant are olivaceous under the microscope and elliptical in shape—features usually considered as characteristic for the *Xerocomus* group. The name is applied to this collection only tentatively.

BOLETUS UNICOLOR ? Frost

Pileus 6–8 cm. in diameter, convex to plane, slightly viscid when moist, subglabrous, "Mustard Yellow" or "Primuline Yellow," darker with age. Tubes 3–6 mm. long, rather short when young, concolorous with the cap, becoming "Ochraceous Buff," free; pores

0.5–1 mm. across, concolorous with the tubes, angular. Stipe 7–9 cm. long, 13–20 mm. thick, concolorous with the cap, glabrous, subequal. Flesh pale "Amber Yellow," unchanging.

Spores olivaceous under the microscope, ellipsoid, $9\text{--}12 \times 5\text{--}6 \mu$ ($11 \times 5 \mu$).

Oct. 13, 1938, Shishan, Kunming, C. C. Cheo (7716).

Boletus rugosellus sp. nov.

Pileo 4.5–11 cm. lato, convexo dein plano-convexo, sicco, glabro et nitido, primitus rugoso, mox laevi, "Tawny," tubulis 5–7 mm. longis, "Green Yellow," ochrascentibus, adnatis v. liberis; poris 2 in uno mm., "Green Yellow," angularibus; stipite 8–15 cm. longo, 7–14 mm. crasso, subaequali v. sursum attenuato, apice flavido, infra "Shell Pink" v. "Japanese Rose," obscuriore brunneo-striato, interdum albo-pubescenti; carne flavida, immutabili; sporis olivaceis, ellipticis, $9\text{--}12(17) \times 4.5\text{--}5.5 \mu$ ($11 \times 4.5 \mu$).

Pileus 4.5–11 cm. in diameter, convex then nearly plane, dry, glabrous and shining, at first rugose, becoming smooth, "Tawny." Tubes 5–7 mm. long, "Green Yellow," free; pores 0.5 mm. across, concolorous with the tubes, simple, angular. Stipe 8–15 cm. long, 7–14 mm. thick, yellowish at the apex, "Shell Pink" downward, streaked with brownish fibrils, sometimes tinged "Japanese Rose" and covered with white down, subequal, slightly tapering upward. Flesh whitish to yellowish, unchanging.

Spores olive under the microscope, elliptical, $9\text{--}12(17) \times 4.5\text{--}5.5 \mu$ ($11 \times 4.5 \mu$).

July 22, 1942, Shishan, Kunming, W. F. Chiu (7872), **Type**. Under *Keteleeria Evelyniana*; Sept. 1942, the same locality (8245).

This fungus is close to *B. recedens*, but of different color. The longer spores of the present plant are also characteristic.

BOLETUS RUBROPUNCTUS Peck

Pileus 3.5 cm. in diameter, convex, "Sanford Brown" to "Burnt Sienna," subglabrous, dry. Tubes up to 7 mm. long, "Pinard Yellow," unchanging, free; pores small, about 0.5 mm. across, roundish, concolorous with the tubes. Stipe 8 cm. long, 3.5 cm. thick, "Baryta Yellow," punctate with reddish brown elements at the apex, streaked with reddish-brown fibrils downward, subventricose, tapering at the base. Flesh yellowish, unchanging.

Spores pale greenish olive under the microscope, long-elliptical, $11\text{--}14 \times 4\text{--}4.5 \mu$ ($14 \times 4.5 \mu$).

July 12, 1943, Shishan, Kunming, W. F. Chiu (8204).

Boletus Taianus sp. nov.

Pileo 5-7 cm. lato, semigloboso dein plano-convexo, glabro, "Pale Olive Buff"; tubulis 5-8 mm. longis, flavis, fractis caerulescentibus, adnatis; poris 1 mm. latis, "Ox-blood Red" v. "Carmine," angularibus; stipite 6-8 cm. longo, 15-20 mm. crasso, leniter sursum attenuato, supra "Begonia Rose," infra brunneo, reticulato; carne firma, albida, fracta pilei caerulescente, stipite punicea; sporis pallidiore olivaceis v. hyalinis, ellipticis v. subfusiformibus, $8-9 \times 3-4 \mu$ ($9 \times 3 \mu$).

Pileus 5-7 cm. in diameter, hemispherical becoming plano-convex, glabrous, "Pale Olive Buff"; margin usually wavy. Tubes 5-8 mm. long, yellow, turning blue when bruised, adnate; pores "Ox-blood Red" or dark "Carmine," about 1 mm. across, rather angular. Stipe 6-8 cm. long, 15-20 mm. thick, brown at the basal portion, "Begonia Rose" upward, reticulate at the upper half of the stipe, slightly tapering upward. Flesh firm, white, turning bluish in the cap and pinkish in the stipe, rather fibrous.

Spores pale olivaceous or rather hyaline under the microscope, elliptical to subfusiform, $8-9 \times 3-4 \mu$ ($9 \times 3 \mu$).

July, 1938, C. C. Cheo from Kunming market (8308), **Type**.

B. Satanus is a species closely related to the present fungus, but the non-bulbous stipe and the grayish cap which is never tinged greenish, are two external characters for separating *B. Taianus* from *B. Satanus*. The small spores of *B. Taianus* are also characteristic.

Boletus sinicus sp. nov.

Pileo 9-11 cm. lato, pulvinato, fibrilloso-squamoso, "Garnet Brown"; tubulis 4 mm. longis, concavis, uncinatis, "Maize Yellow," fractis caerulescentibus; poris minutis, 1-2 in uno mm., "Ox-blood Red." Stipite 8-9 cm. longo, 13-36 mm. crasso, aequali, basi leniter bulboso, pileo concolore, apice flavo, reticulato; carne alba, stipiti flavida, fracta caerulescente; sporis pallidiore olivaceis, breviter ellipsoideis, $7.5-11 \times 4.5-5.5 \mu$ ($9 \times 4.5 \mu$).

Pileus 9-11 cm. in diameter, pulvinate, fibrillose scaly, "Garnet Brown"; margin paler in color and wavy. Tubes 4 mm. long, concave, uncinata, "Maize Yellow," turning blue when cut; pores small, less than 0.5 mm. across, "Ox-blood red." Stipe 8-9 cm. long, 13-36 mm. thick, equal, slightly dilated at the base, concolorous with the cap or paler, yellow at the apex, reticulate with prominent red veins. Flesh white, yellowish, turning blue when cut.

Spores light olivaceous under the microscope, short ellipsoid, $7.5-11 \times 4.5-5.5 \mu$ ($9.5 \times 4.5 \mu$).

July 1938, F. L. Tai from Kunming market (8035), **Type.**

This species is very close to *B. Frostii* and *B. magnificus*. The present fungus differs however from the American species in the scaly pileus, and from *B. magnificus* in the completely reticulate stipe and shorter spores.

BOLETUS QUELETII Schulz.

Pileus 5–8 cm. in diameter, hemispherical to convex, dry, glabrous, shining, "Cinnamon Brown" to "Brick Red" or "Auburn." Tubes 5–6 mm. long, yellow, sinuate-free; pores pale "Brazil Red" becoming "Carrot Red" tinged yellow, minute, less than 0.5 mm. across, changing to green when bruised. Stipe 6–9 cm. long, 3–5 cm. thick, subequal or bulbous, yellow at the apex, pinkish downward, becoming brown with age, slightly reticulate with red veins on the upper portion. Flesh yellow when young, changing to green in the upper portion and lilac in the lower. Flesh of maturing sporophores white in the cap and yellow in the stipe, changing slowly to green where bruised.

Spores olivaceous under the microscope, elliptical, $10-16 \times 5-6 \mu$ ($11 \times 5 \mu$).

July 8, 1942, Tapugi, Kunming, W. F. Chiu (7875 and 7847); Aug. 1938, W. F. Chiu from Chengtu market (Chiu 54).

Boletus magnificus sp. nov.

Pileo 5–11 cm. lato, pulvinato dein convexo, "Nopal Red," "Ox-blood Red" v. "Garnet Brown," sicco, interdum "Coral Red," tomentoso, mox glabrescenti; tubulis 7–12 mm. longis, "Lemon Chrome," aetate ochrascentibus, fractis caerulescentibus, liberis; poris minutis, 1–2 in uno mm., "Brazil Red," aetate "Bittersweet Orange," rotundatis v. plus minus angularibus; stipite 5–15 cm. longo, 2–6 cm. crasso, subaequali, basi leniter bulboso, supra "Capucine Yellow," infra pileo concolore, rubro-punctato v. striato, apice raro reticulato, centrico v. excentrico; carne "Pinard Yellow," caerulescente; sporis pallidiore olivaceis, ellipticis, $9-13 \times 4-6 \mu$ ($11 \times 4-5 \mu$).

Pileus 5–11 cm. in diameter, pulvinate or convex, "Nopal Red," "Ox-blood Red" or "Garnet Brown," dry, sometimes tinged "Coral Red" or becoming paler, tomentose, sometimes becoming glabrous; margin incurved when young then straight, sometimes wavy. Tubes 7–12 mm. long, "Lemon Yellow" to "Lemon Chrome," becoming ochraceous with age, changing to blue when cut, free; pores minute, 0.5–1 mm. across, "Brazil Red," becoming "Bittersweet Orange" with age, roundish or more or less angular. Stipe 5–15 cm. long, 2–6 cm. thick, "Capucine Yellow" in the upper

part, concolorous below, punctate with red-dotted elements or streaked with red fibrils, or rarely reticulate with red veins at the apex, subequal or slightly bulbous at the base, central or excentric. Flesh "Pinard Yellow," turning immediately blue from outside inward, especially in the cap, when exposed to the air.

Spores pale olivaceous under the microscope, elliptical to fusi-form-elliptical, $9-13 \times 4-6 \mu$ ($11 \times 4.5 \mu$).

July 6, 1938, C. C. Cheo from Kunming market (7886); Aug. 1941, Tapugi, Kunming, F. L. Tai (7893, 7693, 7712 and 7719, **Type**); Aug. 3, 1938, S. T. Chao from Kunming market (7727); Aug. 1942, W. F. Chiu (7744); July 9, 1943 (8207); June 16, 1942, Miaokaotze, Kunming, W. F. Chiu (7866). Under *Pinus Armandii*.

This red bolete is very close to *B. Queletii* and is also easily confused with *B. Frostii* and *B. sinicus*, but it is never entirely reticulate on the stipe as in the case of *B. sinicus*. The color of the cap and of the stipe changes to dirty brown or even blackish on touch, and the color of the pores is usually very dark "Brazil Red." The intensity of the red color fades as the age advances. The orange color of the pores in the later stages of development is likely to lead one to consider such stages as a distinct variety.

Boletus subsplendidus sp. nov.

Pileo 2.5-6 cm. lato, convexo dein plano, sicco, minute tomentoso, "Burnt Sienna" v. "Bay," saepe ad marginem flavo-maculato, tacto caerulescenti; tubulis primitus brevissimis, aetate usque ad 7 mm. longis, "Empire Yellow," adnate subdecurrentibus; poris minutis, concolore, fractis caerulescentibus; stipite 6-9 cm. longo, 15-20 mm. crasso, subaequali v. subventricoso, striato e pubescenti, interdum reticulato, tacto brunnescenti; carne flava, caerulescente; sporis olivaceis, ellipsoideis, $9-12 \times 4-5 \mu$ ($9 \times 4.5 \mu$).

Pileus 2.5-6 cm. in diameter, convex to nearly plane, dry, minutely tomentose, "Burnt Sienna" to "Bay," usually maculate or bordered with "Empire Yellow" at the marginal region, turning bluish when bruised. Tubes very short when young, up to 7 mm. long at maturity, "Empire Yellow," adnate-subdecurrent; pores minute, concolorous with the tubes, turning blue when exposed to the air. Flesh yellow, changing to bluish in the cap and greenish in the stipe. Stipe 6-9 cm. long, 15-20 mm. thick, "Empire Yellow," usually lateritious in the lower part, subventricose or subequal, streaked and pubescent, sometimes obscurely reticulate, turning brown when bruised.

Spores olive under the microscope, ellipsoid, $9-12 \times 4-5 \mu$ ($9 \times 4.5 \mu$).

July 12, 1942, W. F. Chiu, Miaokaotze, Kunming (7876), **Type**. In mixed woods of *Pinus yunnanensis*, *Keteleeria Evelyniana* and *Lithocarpus* sp.

This plant is close to *B. splendidus* Mart. except that the flesh of the former never changes to red or purple when exposed to the air, and that the yellow stipe is streaked, pubescent and occasionally obscurely reticulate.

BOLETUS ALBIDUS Roq.

Pileus 6-16 cm. in diameter, hemispherical becoming convex, usually slightly depressed at the disk, dry, glabrous, pale "Chestnut Brown" or "Warm Sepia," sometimes even "Avellaneous" or "Wood Brown"; margin involute when young. Tubes very short in young sporophores, about 4-5 mm. long in maturing ones, "Pinard Yellow" turning olivaceous with age, sinuate to subdecurrent; pores minute, 0.5 mm. (or less) across, yellow, simple, roundish at the basal portion, distinctly reticulate with yellow veins, tinged reddish at the base, equal or ventricose. Flesh straw-yellow or whitish, changing immediately to blue when exposed to the air, sometimes changing to reddish in the lower part of the stipe, rather fibrous.

Spores pale olive under the microscope, elliptical, $8-12 \times 3-5 \mu$ ($9 \times 4 \mu$).

June to Aug. 1941, from Kunming market (5475, 5483, 7690, 7696, 7710, 7711, 7722, 7885 and 8202).

Gilbert maintains that *B. albidus* Roq. and *B. candidus* Fr. are synonymous with *B. vitellinus* Pers. Nevertheless, there is no valuable diagnosis of the species of Persoon. It seems better to keep the name *B. albidus* Roques. The present writer is of the opinion that this species might not be a synonym of *B. radicans* Pers. ex Fr. Kallenbach's illustration is evidently different from Roques' original diagnosis. Variation of the color and the shape of the plant has been observed in the Yunnan collections. The young sporophores are usually darker in color but become much paler with age. The shape of the cap varies from convex to plano-convex with or without umbilicus. The margin of the cap is incurved when young and becomes straight with age.

BOLETUS SPECIOSUS Frost

Pileus 5-16 cm. in diameter, pulvinate becoming convex to plano-convex, dry, "Light Salmon Orange," "Capucine Orange," "Carnelian Red," "Old Rose," sometimes fading to "Onion Skin Pink," tomentose or becoming glabrous; margin entire, incurved when young, becoming straight later, usually wavy. Tubes 5-10 mm. long, "Lemon Yellow," changing to blue when bruised, uncinuate to free; pores 0.5-1 mm. across, concolorous with the tubes, simple, round, becoming brownish with age. Stipe 4-11 cm. long, 1-4 cm. thick, "Eugenia Red," yellowish at the apex, "Garnet Brown" or "Brazil Red" at the base, innately reticulate with "Garnet Brown" veins throughout the stipe or only at the apex, or only streaked with "Garnet Brown" veins or fibrils, equal or attenuated toward the apex, usually dilated, central or subexcentric. Flesh solid, pale yellow, turning slowly blue-green when exposed to the air, reddish in the basal portion of the stipe.

Spores fusiform-elliptical, olivaceous under the microscope, 9-11(14) \times 4-5 μ (9 \times 5 μ).

July to Aug. 1941, from Kunming market, F. L. Tai et C. C. Cheo (7732, 7746, 7737, 7740, 7757, 7751 and 7844).

The variation of the color of this plant is rather extensive, usually from "Light Salmon Orange" to "Old Rose" through a series of intergradations. The Yunnan form has a red stipe which is sometimes yellow only at the apex (identical with the illustration in Icon. Farlow. Pl. 82). The reticulated veins on the stipe are red and very distinct but may be lost by handling or smearing. It is sometimes difficult to separate the very old specimens of this species from those of *B. sanguineus* With. merely by their external characters.

BOLETUS REGIUS Kromb.

Pileus 8 cm. in diameter, convex, dry, glabrous, pale "Pinkish Buff" to "Hydrangea Pink," becoming brown when bruised. Tubes 8 mm. long, yellow becoming greenish, uncinuate; pores minute, less than 0.5 mm. in diameter, yellow, becoming greenish when bruised. Stipe 10 cm. long, 3 cm. thick, cylindrical, subbulbous at the base, yellow, reddish brown at the base, distinctly reticulate. Flesh pale yellow in the cap, whitish in the stipe, turning reddish where tunneled by worms, rather compact.

Spores pale "Cendre Green" under the microscope, fusiform-elliptical, usually 3-guttulate, $10-12(13) \times 4-5 \mu$ ($11 \times 4.5 \mu$).

June 11, 1942, W. F. Chiu, from Kunming market (7846).

BOLETUS SANGUINEUS With.

Pileus 7-8.5 cm. in diameter, hemispherical, dry, "Victoria Lake" to "Maroon Red," tomentose, rimose-areolate, crevices yellow; margin more or less wavy. Tubes 6 mm. long, uncinatate, yellow, turning bluish green; pores roundish, 0.5-1 mm. across, concolorous with the tubes. Stipe 4.5 cm. long, 12-22 mm. thick, subequal, slightly dilated at the middle, reticulate in the lower part with dark veins and in the upper with yellow veins, yellow near the apex, "Shrimp Pink" below, brownish-black at the base. Flesh yellow, changing to blue-green.

Spores cremeous in mass, hyaline under the microscope, oblong-elliptical, $9-15 \times 4-5 \mu$ ($11 \times 5 \mu$).

Summer, 1939, Kunming market (7732 and 7746).

In general, the reticulation on the stipe is limited only to the upper portion, but one specimen was found with veins extending distinctly down to the base.

BOLETUS ORNATIPES Peck

Pileus 2-8 cm. in diameter, pulvinate becoming convex, dry, minutely tomentose when young, becoming glabrous with age, "Saccardo Olive" to "Olive Brown." Tubes 6-10 mm. long, "Lemon Chrome," adnate to subdecurrent; pores simple, roundish, concolorous with the tubes, unchanging. Stipe 4-11 cm. long, 1-3 cm. thick, yellow, reticulate with concolorous veins, subequal, stuffed. Flesh yellow, unchanging, rather fibrous.

Spores slightly yellowish under the microscope, elliptical to ellipsoid, $8-12 \times 4-5 \mu$ ($9 \times 5 \mu$).

June 30, 1941, C. C. Cheo, Miaokaotze, Kunming (7729); Sept. 14, 1941, Tapugi, C. H. Hung (7464); June 25, 1942, Miaokaotze, W. F. Chiu (7861). In coniferous woods (the dominant species of the woods is *Keteleeria Evelyniana*).

Lohwag has studied some specimens collected at Likang, Yunnan, and found that the Likang plant is distinct from both of the American species, *B. retipes* B. et C. and *B. ornatipes* Peck. It is said that the Likang plant has a glabrous cap and large spores (13-

15 \times 5-6 μ) (Symbolae Sinicae II: 57, 1937). In addition, he is of the opinion that *B. retipes* B. et C. is not synonymous with *B. ornatipes*. The present writer agrees with Lohwag in that *B. retipes* is distinct from *B. ornatipes*, but is skeptical on the validity of the new species *B. Kauffmanii*. Although the present writer has not been able to visit the Northwest of Yunnan where the type specimen of Lohwag was collected, yet in the vicinity of Kunming specimens of such type are abundant and have been collected annually. As a result of comparative studies on the characters of the plant in various stages of development, it has been found that a certain stage of growth of the plant is almost identical with Lohwag's new species. In general, the cap of this fungus is minutely tomentose when young and becomes glabrous with age, and the spores are shorter than those of Lohwag's species and those of American species, but are little broader than the latter. In Lohwag's diagnosis there is no definite description of the color of the stipe. As to flesh, Lohwag did not mention whether or not his description is based on notes of fresh specimens supplied by the collector. The color of the flesh, so far as he recorded, is "Rosaceo-griseola." Such color can be found in our dried specimens. In fresh specimens, the flesh is always yellow. The writer therefore hesitates to recognize the validity of *B. Kauffmanii* as a distinct species.

BOLETUS RIMOSELLUS Peck

Pileus 6-10 cm. in diameter, hemispherical then convex to plane, "Ochraceous Buff," "Buckthorn Brown" or "Cinnamon," maculate with brown elsewhere, subglabrous, often cracked at the margin and rimose-areolate on the surface. Tubes up to 10 mm. long, yellow, turning ochraceous with age, sinuate-free; pores minute, 2-3 per mm., concolorous with tubes. Stipe 5.5-9 cm. long, 1-2 cm. thick, "Light Pinkish Cinnamon," reticulate with brown veins, cuticle cracked and reflexed, becoming scaly, equal or tapering upward, rather thick, stuffed. Flesh white, unchanging.

Spores dark olive under the microscope, elliptical, 2-3 guttulate, 9-14 \times 4.5-5.5 μ (14 \times 4.5 μ).

Summer, 1938, from Kunming market (7891).

BOLETUS GERTRUDIAE Peck

Pileus 3–8 cm. in diameter, convex or slightly depressed, dry, glabrous, "Xanthine Orange" to "Amber Brown." Tubes 5–12 mm. long, yellow, adnate to uncinatate, unchanging; pores concolorous with the tubes, about 1 mm. across, roundish. Stipe subconcolorous in the upper part and much paler in the lower part, punctato-pubescent, becoming glabrous, partially or completely reticulate with brownish veins. Flesh white, unchanging.

Spores dark olive under the microscope, oblong to subfusiform, $14-17 \times 5-6 \mu$ ($15 \times 5 \mu$).

Aug. 28, 1938, Chunhotze, Tali, C. C. Cheo (7784).

The reticulation of the stipe is not a constant character in this species. According to Peck's original description, the stipe is said to be smooth, but Snell reported reticulation on the stipe of his collection (Mycologia 28: 17, 1936). The Yunnan form is rather close to Snell's collection.

BOLETUS EDULIS Bull.

Pileus 5–9 cm. in diameter, hemispherical to convex, becoming plane, dry, minutely tomentose, becoming glabrous, "Honey Yellow," "Amber Brown" to "Antique Brown," usually paler or darker than the colors described. Tubes 10–15 mm. long, white when young, becoming "Antimony Yellow," "Old Gold" or "Buff Citrine" with age, uncinatate, depressed around the stem; pores 0.5–1 mm. across, concolorous with the tubes or light "Ochraceous Salmon," roundish, simple. Stipe 6–9 cm. long, 3–4 cm. thick, "Light Pinkish Buff" to "Wood Brown," distinctly reticulate with white veins, subequal, usually slightly dilated at the base or bulbous, rarely tapering downward. Flesh white, tinged pinkish in the cap.

Spores pale "Viridine Green" under the microscope, oblong-elliptical, $(9)11-14(16) \times 4-5 \mu$ ($12 \times 5 \mu$).

July to Aug. 1938, from Kunming market (5478, 5575, 5579, 5583, 7750, 7756, 7841 and 7887); July to Aug. 1938, from Tapugi market (7733, 7745 and 7753); Aug. 7, 1942, Miaokaotze, Kunming, W. F. Chiu (7855). In coniferous woods.

The flesh of the Yunnan form is usually white and unchanging, but occasionally colored slightly pinkish. The form of the stipe is not necessarily always bulbous, but varies from slender to ventricose and bulbous, and even to tapering downward.

BOLETUS EXIMIUS Peck

Pileus 9–11 cm. in diameter, pulvinate or hemispherical becoming plane, "Hay's Maroon," "Diamine Brown" or "Hessian Brown," glabrous in the central portion, slightly tomentose at the margin. Tubes 8–14 cm. long, "Citrine," becoming ochraceous or subconcolorous with the cap, sinuate, depressed around the stem; pores 0.5–1 mm. across, "Vinaceous Purple" becoming "Russet Vinaceous," simple, roundish. Stipe 4–10 cm. long, 1–3 cm. thick, purplish, brownish in the lower portion, punctate with dark-purple or violet dots, subequal or ventricose. Flesh firm, turning lilac and punctate with dark-purple glandules in the cap, streaked with violet fibrils in the stipe.

Spores olivaceous under the microscope, narrowly elliptical to subfusiform, $11-17 \times 4-5 \mu$ ($8 \times 4 \mu$).

According to Peck's diagnosis, the tubes are said to be concolorous with the cap. In the Yunnan form, the tubes are at first citrine and then become concolorous with the cap.

Boletus brunneissimus sp. nov.

Pileo 3–9 cm. lato, hemisphaerico dein convexo, sicco, tomentoso, "Raw Umber," interdum rimoso-areolato; tubulis 10 mm. longis, aetate olivaceis, sinuatis v. liberis; poris minutis, 2–3 in uno mm. primitus "Carob Brown" mox "Brussels Brown" v. "Xanthine Brown" deinde "Mars Yellow," rotundis; stipite 4–9 cm. longo, 10–25 mm. crasso, "Light Pinkish Cinnamon" aetate "Nopal Yellow" v. "Chamois," punctato-striato, subaequali, deorsum attenuato; carne flava, caerulescente, stipite alba, fibrillosa, rufescente mox cyanescente; sporis olivaceis, ellipsoideis, $9-12 \times 4-5 \mu$ ($11 \times 5 \mu$).

Pileus 3–9 cm. in diameter, hemispherical then convex, dry, tomentose, "Raw Umber," darker when dry, sometimes rimose-areolate. Tubes about 1 cm. long, yellow, becoming olivaceous, sinuate or free, slightly depressed around the stipe; pores minute, about 0.5 mm. across, at first "Carob Brown" then "Brussels Brown," finally fading to "Xanthine Brown" or "Mars Yellow." Stipe 4–9 cm. long, 10–25 mm. thick, "Light Cinnamon," becoming "Nopal Yellow" or "Chamois" with age, densely covered with dark-brown-dotted elements and fibrils but smooth at the apex, subequal, occasionally tapering downward. Flesh yellow in the cap changing to blue, white and fibrous in the stipe becoming pinkish, greenish and pinkish in the basal portion of the stipe.

Spores olivaceous under the microscope, elliptical, $9-12 \times 4-5 \mu$ ($11 \times 5 \mu$).

June 21, 1941, Miaokaotze, C. C. Cheo (7704 and 7713); June 25, 1942, the same locality, W. F. Chiu (7864, **Type**); 1938 to 1941, from Kunming market (7752, 7724, 7718, 7892 and 7899); July 26, 1938, Mt. Omei, Szechwan, W. F. Chiu (7687). In mixed woods of *Keteleeria*, *Pinus* and *Castanopsis*.

This plant differs from *B. umbrinus* in the yellow tubes, dark brown pores, "Raw Umber" cap and the distinctly punctate and striate stipe. In our collections, two forms have been observed: the sterile and the fertile. The sterile form possesses very short tubes usually less than 1 mm. in length, and therefore might be mistaken for a *Gyrodon*. If it were a case of immature fruiting bodies instead of sterile ones, the size and shape of the fruiting bodies would be more or less different from the mature ones. Nevertheless, there is no difference between them. Another evidence that the sterile form is really sterile instead of being immature, is the occasional tessellate crackings on the cap.

STROBILOMYCES Scop.

Strobilomyces glabriceps sp. nov.

Pileo 10 cm. lato, convexo, "Carob Brown," glabro, rimoso-areolato; tubulis usque a 25 mm. longis, adnatis, decurrentibus; poris amplis, 4-5 mm. latis, alveolatis, pileo subconcolore; stipite 12 cm. longo, 15-30 mm. crasso, deorsum attenuato, basi bulboso, "Russet Brown," striato ac furfuraceo; sporis fusco-brunneis, globosis, asperulis, reticulatis, 9-12(11) μ .

Pileus 10 cm. in diameter, convex, "Carob Brown," glabrous, rimose-areolate. Tubes 20-25 mm. long, decurrent; pores 4-5 mm. across, "Mikado Brown," decurrent on the stipe, angular or comb-like, rather large. Stipe 12 cm. long, 15-30 mm. thick, "Russet Brown," streaked and furfuraceous, usually tapering toward the base and slightly bulbous at the base.

Spores dark brown under the microscope, globose to subglobose, reticulate, 9-12(11) μ .

June 7, 1938, Shishan, Kunming, T. K. Yien (8034), **Type**.

The spores of this plant are not readily distinguishable from those of *S. floccopus*, but the glabrous and rimose-areolate cap and the very decurrent tubes are distinct characters.

STROBILOMYCES FLOCCOPUS (Vahl. ex Fr.) Karsten

Pileus 2.5–8 cm. in diameter, hemispherical to convex, grayish brown, covered with dark brown, erect, recurved, or pyramidal scales, usually appendiculate on the margin. Veil membranous, grayish. Tubes 10–25 mm. long, ventricose, adnate, grayish becoming black; pores 1.5–2.5 mm. across, purplish gray turning black with age. Stipe 4–14 cm. long, 8–20 mm. thick, concolorous with the cap, fibrous-scaly. Flesh brownish to lilac in the stipe, often paler in the cap.

Spores globose to subglobose, dark brown under the microscope, reticulate, 8–11 (9) μ .

July to Sept. 1940, F. L. Tai, C. C. Cheo, S. T. Chao, from Kunming (5471, 5474, 7709, 7726, 7767, 7871, 7878 and 7879); 1938, C. C. Cheo, Tali, (7779); W. F. Chiu, 1938, Mt. Omei, Szechwan (7687). Usually in mixed woods of *Quercus variabilis* and *Pinus yunnanensis*. In one case, the present writer encountered this species under bamboo.

STROBILOMYCES RETISPORUS (Pat. et Bak.) Gilb.

Pileus 6–10 cm. in diameter, convex to plano-convex, sometimes slightly depressed at the center, dry, "Nopal Red" to "Garnet Brown," sometimes much paler and even tinged pinkish, tomentose becoming glabrous. Tubes up to 15 mm. long, "Light Dull Green Yellow," free to adnate or depressed around the stipe according to age; pores about 1 mm. across, concolorous with the tubes, roundish, compound. Stipe 7–14 cm. long, 12–20 mm. thick, "Lemon Yellow" to "Ochraceous Buff," tinged "Ochraceous Salmon" in the basal portion, reticulate with yellow veins, slightly tapering upward. Flesh "Pale Pinard Yellow," changing to reddish somewhere in the stipe especially at the base, sometimes changing to bluish somewhere in the upper part of the stipe.

Spores brown under the microscope, broad-elliptical, reticulate, 12–19 \times 8–9 μ (16 \times 8 μ).

Aug. 7, 1942, Miaokaotze, Kunming, W. F. Chiu (7865); July 12, 1943, Shishan, Kunming (8004). In woods of *Pinus yunnanensis* and *Keteleeria Evelyniana*.

The cap of this plant varies from tomentose to glabrous while in the original diagnosis of Patouillard and Baker the cap is said to be glabrous.

BOLETELLUS Murr.

BOLETELLUS ANANAS (Curt.) Murr.

Pileus 2-3.5 cm. in diameter, hemispherical to convex, "Tawny Olive" with reddish brown squamules, minutely tomentose, usually rimose-areolate, becoming "Snuff Brown" and glabrous at the center; the veil formed by the extruding cuticle of the cap persistently applied to the apex of the stipe, forming a sleeve. Tubes 3-4 mm. long, "Light Cadmium Yellow," depressed around the stipe, usually changing to blue when bruised; pores 1 mm. across, concolorous with the tubes, angular, simple. Stipe 4-5 cm. long, 5-10 mm. thick, "Light Pinkish Cinnamon," "Vinaceous Cinnamon" at the base, fibrillose to subglabrous, subequal or slightly tapering upward. Flesh white, turning blue when exposed to the air.

Spores ochraceous under the microscope, elliptical to broad elliptical, longitudinally striate, $12-21 \times 6-8 \mu$ ($17 \times 8 \mu$).

Sept. 15, 1938, C. C. Cheo, Chichushan, Binchwan (7805). Under *Pinus Armandii*.

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EFFECT OF NUTRITION ON GROWTH AND MORPHOLOGY OF THE DERMATOPHYTES.

1. DEVELOPMENT OF MACROCONIDIA IN TRICHOPHYTON RUBRUM

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(WITH 2 FIGURES)

THE IDENTIFICATION OF THE DERMATOPHYTES. The dermatophytes were originally distinguished from other fungi by their pathogenicity. In parasitic life they flourish only in keratin containing tissues, the skin and appendages. There are three recognized genera which were at first differentiated by the clinical picture for which each was responsible, and to some extent by their appearance in tissues. It is now recognized, however, that the morphology and biological characteristics of the fungi themselves form a sounder basis for identification.

Dermatophytes as a family show some general similarity in colony form and microscopic structure. It is, however, difficult to define them botanically. All dermatophytes produce round or pyriform microconidia often attached to the mycelium by a collarette, but hardly distinctive enough to separate the dermatophytes from other forms. The majority produce multicellular macroconidia (fuseaux) which are more distinctive.

The three genera are best defined by the character of these macroconidia (Emmons 1). Species can be distinguished only by a combination of characteristics such as minor differences in macroconidia, differences in form and arrangement of the microconidia, and by mycelial structures known as nodular organs, spirals, "chandeliers" and "antlers." The form of colony and pigments produced are also important.

Identification of dermatophytes is often difficult because colony form, pigmentation and spore production vary greatly under dif-

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ferent conditions of culture. Some strains fail to produce on any known medium structures considered characteristic of the genus and species with which they conform in other respects. Many show minor variations from type strains which are difficult to evaluate by present methods.

INFLUENCE OF NUTRIENTS ON MORPHOLOGY. Sabouraud (2) in his monumental work, *Les Teignes*, published in 1910, emphasized the influence of culture media on growth characteristics. He devised two "proof media" and a "conservation medium" prepared from specified brands of peptone, crude glucose and crude maltose, and published exact descriptions of the gross and microscopic characteristics of numerous species when grown on these media.

Subsequent workers used these same media for identification of their cultures. When, after the first world war, the French maltose and peptone which Sabouraud used were no longer available, substitutes had to be found. Thus Weidman and Spring in 1928 (3) published a comparison of ringworm culture media. They decided that Fairchild's peptone was a fair substitute for the French peptone, though not perfect. This with crude American dextrose could be used to produce a "standard medium." About the same time, Hodges (4) compared cultures of ringworm fungi on Sabouraud's Proof medium and on media prepared with American peptones and sugars. He proposed a standard medium containing Fairchild's peptone and American granular dextrose. It was thought that if we could find a suitable standard medium readily obtainable by all workers, we would greatly advance our knowledge of these fungi. This has been more or less realized in the production of dried powders by the commercial houses. While it is advantageous to have the various workers in the field using comparable media, those now in use do not seem the final answer to the problem of increasing our understanding of these forms.

The above mentioned media were devised chiefly to obtain typical colony forms, but other factors such as pigment production and especially the microscopic morphology are more important in the identification of the dermatophytes. It has long been known that the media usually used for the isolation of fungi or for the

production of characteristic colonies, such as Sabouraud's honey or dextrose agars, are not satisfactory for the study of microscopic characteristics. Media such as corn meal, potato or wort agar are commonly used for this purpose. Realizing this inadequacy of the media in common use, Langeron (5) and his co-workers introduced the use of the so-called natural media, such as rice, barley and wheat grains. These were found more favorable for the production of spores.

The same medium will not, however, serve the same purpose for each species. Different species have their special requirements and preferences, *e.g.*, as shown by Lewis and Hopper (6), the presence of dextrose favors the production of diffusible pigment by *Microsporum canis* and *Trichophyton rubrum* but not by *Microsporum audouini* or *Trichophyton mentagrophytes*.

In the genus *Microsporum* there are three well recognized species, *M. canis*, *M. gypseum* and *M. audouini*. On all the usual media the two former species produce the characteristic macroconidia abundantly, whereas *M. audouini* forms them very rarely. An important contribution was made by Benedek (7) when he discovered that the growth of *M. audouini* on polished rice grains was stimulated by the presence of a bacterium which he named *B. weidmaniensis*, and that in addition to the increased growth, macroconidia characteristic of the genus *Microsporum* were formed. Conant (8) had previously shown that *M. audouini* would not grow on rice grains, whereas *M. canis* grew readily. These facts have been confirmed in our laboratory.

INFLUENCE OF VITAMINS. Recently, Hazen (9) has shown that the addition of yeast extract to rice has the same effect on *M. audouini* as inoculation with *B. weidmaniensis*, *i.e.*, it causes free growth with production of macroconidia. That vitamins may play an important part in the nutrition of fungi has been demonstrated many times. Robbins, MacKinnon and Ma (10) found that their strain of *Trichophyton discoides* suffered complete deficiencies for pyridoxine, i-inositol, and molecular thiamine. This organism and the faviform group in general are slow growing fungi isolated with great difficulty, and only on enriched media. This has been brought out by Fowle and Georg (11) in their recent report of ringworm cases contracted from cattle.

From these cases they were able to isolate *Trichophyton discoides* and *Trichophyton album* on Blood Agar Base (Difco), a heart infusion agar containing Bacto-Tryptose. This group of dermatophytes is undoubtedly one for which the media in general use are deficient to a high degree in the elements essential for their growth. Varying degrees of deficiency exist in other groups also, which, no doubt, accounts for the many variations in growth and spore production. *Microsporum audouini* is probably a deficient organism. *Trichophyton mentagrophytes*, on the other hand, has not shown any deficiencies (Burkholder 12), and it is the one of the dermatophytes that grows most readily and produces the greatest number of characteristic structures and spores.

VARIATIONS AND MUTATIONS. One further complication in the study of these forms is the natural variations and mutations which occur, as well as the so-called pleomorphic degeneration which arises when a strain has been on artificial media for some time. These phenomena are probably influenced by the nature of the medium used. For example, Sabouraud observed that the pleomorphic degeneration is less frequent on the sugar free media. Some of the variations may be due to the presence or absence of certain growth substances or vitamins.

We find, then, that numerous characteristics on which we base our identification of dermatophytes are modified by changes in culture media. As we have previously maintained, no one culture medium is adequate for the recognition of all species. Even in a study of a single species one medium will serve one purpose only. What seems desirable is the optimum medium for the development of each significant characteristic.

THE MACROCONIDIA OF *T. RUBRUM*. Identification of *T. rubrum* (*purpureum*) is usually possible on the basis of its colony form on dextrose-peptone (Sabouraud) or similar agars by the elongated microconidia arising from the sides of long hyphae, and especially by the red to purple or wine-red pigment which it produces on most dextrose-containing media. The colony type varies so much, however, that there is some question as to whether the strains now called *T. rubrum* represent a single species or a group of species or variants. Even the pigment which gives the fungus its name varies in color and intensity and may occasionally fail

to appear on peptone media even if they contain dextrose. Moreover, occasionally a strain which morphologically conforms to the gypsum group develops a rose red pigment easily confused with the pigment of *T. rubrum*.

The macroconidia of *T. rubrum* were pictured by Bang (13) as long, narrow spores with nearly straight, parallel sides, and blunt or pointed ends, and three to ten septa. Most subsequent students of this fungus have noted these peculiar structures which are often described as pencil-shaped. Occasionally the cells of the spore are so rounded that it resembles a chain of chlamydospores. While these macrospores resemble those of the other trichophyta in having thin walls they are not easily mistaken for those produced by any other species of dermatophyte. They seem perhaps the most distinctive feature of the species *T. rubrum*. They are, however, inconstant. Rare strains with a powdery, or finely granular, or even cottony type of growth produce such spores fairly regularly on Sabouraud's honey or dextrose agar whereas they rarely are seen in more downy types. Spring (14) found the addition of ascitic fluid increased the frequency of their production but in our hands many strains failed to produce them on this medium.

In search for a more suitable medium, Blood Agar Base (Difco) was tried, because it had been found a good medium for the isolation and study of the faviform trichophyton group. Ten strains of *T. rubrum* were planted on this medium, and of these eight were found to produce macroconidia in good numbers, one formed only a few, and one failed to produce them. With these encouraging results it was decided to study a larger number of strains. In all, fifty strains have been studied on this and other media.

METHOD. The strains studied were for the most part isolated from patients reporting to the Vanderbilt Clinic. Included in the series also were some isolated by Hopkins and associates from army personnel stationed at Ft. Benning, Georgia. The strains were isolated on Sabouraud's honey or dextrose agar—some on potato-dextrose agar. Transfers were maintained on the dextrose agar, and transferred from this to the medium selected for study.

The cultures were identified by their appearance on potato-dextrose agar as suggested by Edgecombe (15), the pigment production noted and the presence or absence of macroconidia on this as well as on honey and dextrose agar was recorded. They were then transferred to Blood Agar Base (Difco) which is stated to contain the following ingredients per liter:

Beef Heart, Infusion from	500 g.
Bacto-Tryptose	10 g.
Sodium Chloride	5 g.
Bacto-Agar	15 g.

Twenty-three strains were also transferred to sterile moist rice grains. Examinations were made at weekly intervals and it was found that the spores usually appeared in about two weeks. Slides were prepared by teasing apart the material in a drop of sodium hydroxide and adding a cover slip. If spores were not found easily, several slides were studied to verify the results.

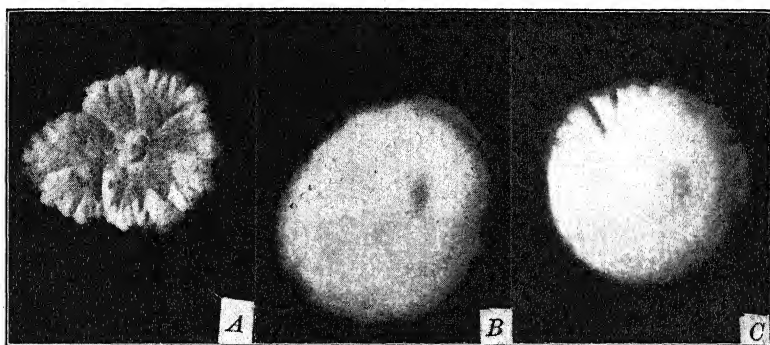


FIG. 1. Colonies of three strains of *T. rubrum* on Blood Agar Base. (A) The flat velvety type of growth. (B) The fluffy type. (C) Downy growth with flat powdery sectors at the border.

RESULTS. On the original honey or dextrose agar tubes only two of fifty strains produced macroconidia.

On rice grains a downy mycelium was visible, but as usual with this type of medium, no characteristic colony was formed. Of twenty-three strains so studied, eight were found to produce macroconidia.

On the Blood Agar Base, *Trichophyton rubrum* produces a compact and restricted type of growth. The colony may be downy,

velvety or fluffy in appearance and of about two to three cm. in diameter at the end of two weeks (FIG. 1). This growth is slow as compared with that on Sabouraud's media and the characteristic pigment is not evident. The surface may be finely wrinkled or corrugated or of the more fluffy type. Microconidia are found in great numbers. Of the fifty strains studied, all but one formed the characteristic long, slender macroconidia, on the Blood Agar Base. Twenty-three of the strains studied formed these spores in great abundance, eight in good numbers, and in seventeen only a few were seen. There was considerable variation in the length of the spores and in the number of cells, which ranged from three to ten, with the average about seven (FIG. 2). The spores form singly at the ends of hyphae or as side branches. Occasionally they were grouped (FIG. 2E). Sometimes they appear to form in the length of the hypha singly or in series and are freed by the breaking up of the hypha (FIG. 2C).

Blood Agar Base differs from Sabouraud's medium in reaction, having a pH of 6.8, in the absence of sugar and in containing beef heart infusion and "tryptose," a meat digest with a higher percentage of proteose than the usual peptones. The pH of the media did not seem to be an important factor. Macroconidia were formed as readily on the Blood Agar Base when the pH was adjusted to 5.5 or 7.4 as on the original which was 6.8.

Results with Beef Heart Infusion. Ten strains were grown from an agar prepared from Bacto-Beef Heart for infusions. This differed from Blood Agar Base in the omission of tryptose. This medium was not favorable for the production of macroconidia as none was found in any of the ten strains tested. Microconidia formed in great abundance.

Results with Tryptose. The same ten strains were inoculated on an agar containing the same amount of Bacto-Tryptose as the Blood Agar Base described above but without Beef Heart Infusion. The growth in this medium resembled that on Blood Agar Base and macroconidia were found in nine of the cultures. This would suggest that it is the Bacto-Tryptose in the Blood Agar Base which makes it so favorable for the production of macroconidia.

DISCUSSION. Forty-nine of the fifty strains tested produced

typical macroconidia on Blood Agar Base, whereas they were found in only two of the strains when grown on Sabouraud's honey or dextrose agar. It would seem that the first mentioned medium is the most favorable yet reported for the production of these

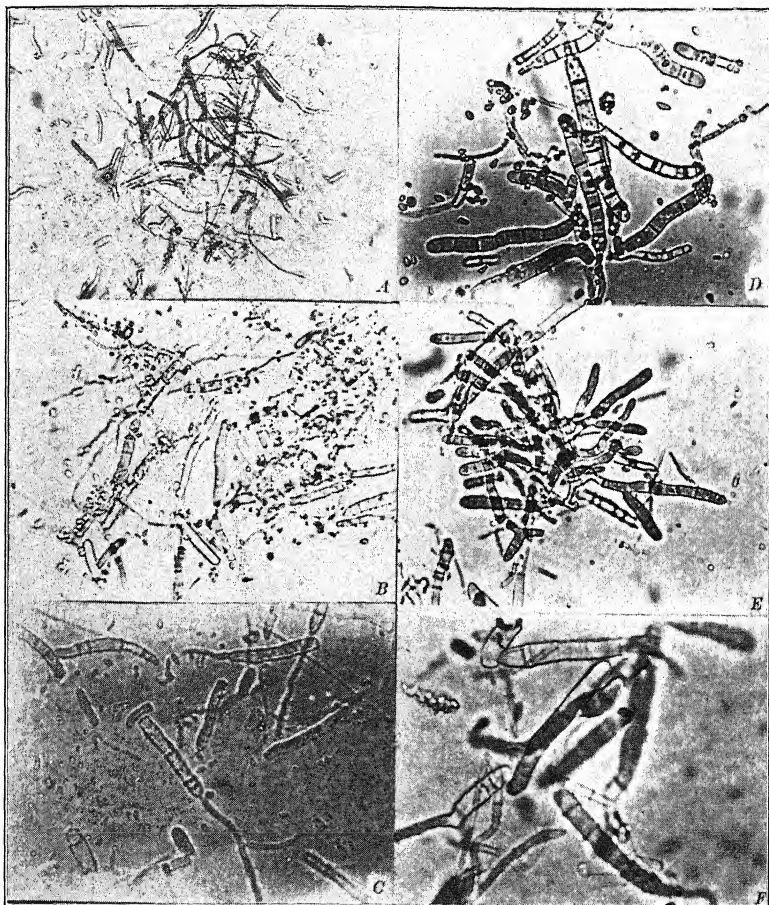


FIG. 2. Macroconidia from three strains of *T. rubrum* on Blood Agar Base. (A) $\times 160$. (B, C, and E) $\times 400$. (D and F) $\times 800$.

characteristic structures. It is believed that its use will facilitate the identification of doubtful strains.

Further studies are in progress as to the relationship of proteoses or other constituents of tryptose to macroconidial formation.

SUMMARY. Of fifty strains of *Trichophyton rubrum* grown on a Heart Infusion Agar plus tryptose, known as "Blood Agar Base," only one failed to produce the characteristic macroconidia. It is believed that tryptose is the ingredient responsible for this spore production.

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THE MEANING OF ARTICLE 57 OF THE INTERNATIONAL RULES

DONALD P. ROGERS

Art. 57. Among Fungi with a pleomorphic life-cycle the different successive states of the same species (*anamorphoses, status*) can bear only one generic and specific name (binary), that is the earliest which has been given, starting from Fries, *Systema*, or Persoon, *Synopsis*, to the state containing the form which it has been agreed to call the perfect form, provided that the name is otherwise in accordance with the Rules. The perfect state is that which ends in the ascus stage in the *Ascomycetes*, in the basidium in the *Basidiomycetes*, in the teleutospore or its equivalent in the *Uredinales*, and in the spore in the *Ustilaginales*.

Generic and specific names given to other states have only a temporary value. They cannot replace a generic name already existing and applying to one or more species, any one of which contains the "perfect" form.

The nomenclature of Fungi which have not a pleomorphic life-cycle follows the ordinary rules.

Examples: The names *Aecidium* Pers., *Caeoma* Link. and *Uredo* Pers. designate different states (aecidiosporic with or without pseudoperidium, uredosporic) in the group *Uredinales*: the generic name *Melampsora* Cast. . . . applied to a genus which is defined by means of the teleutospores, cannot therefore be replaced by the name *Uredo* Pers. . . . since the name *Uredo* is already used to designate a state.¹—Among the *Dothidiaceae* (*Ascomycetes*) a species of the genus *Phyllachora* Nitschke, *P. Trifolii* (Pers.) Fuck. . . . , has an older synonym, *Polythrincium Trifolii* G. Kunze . . . , based on the conidial state of this species: the name *Polythrincium* cannot displace that of *Phyllachora* because it represents an inferior state.—The name *Phoma* Fries emend. Desm. has been given to a group of *Fungi Imperfecti* (*Deuteromycetes*), several members of which have been recognized as the spermogonial state of species of the genus *Diaporthe* (*Valsaceae, Ascomycetes*): thus *Phoma Ailanthi* Sacc. belongs to *Diaporthe Ailanthi* Sacc., *Phoma alnea* (Nitschke) Sacc. to *Diaporthe alnea* Fuck., *Phoma detrusa* (Fries) Fuck. to *Diaporthe detrusa* Sacc. etc. But the perfect state of many species of the "genus" *Phoma* is not known and in some cases probably does not exist: hence the practical necessity for retaining the name *Phoma* to designate the group of *Fungi Imperfecti* in question (Briquet et al. 1935: 17-18).

¹ The original French text, which is here the authoritative one (Briquet 1912: vi, vii; Briquet et al. 1935: viii), reads, ". . . le nom d'*Uredo* est déjà en usage pour désigner un état imparfait" (Briquet 1912: 28; Briquet et al. 1935: 45). Obviously the word "imperfect" was accidentally dropped from the English translation, which should read ". . . the name *Uredo* is already used to designate an imperfect state."

The Article here quoted is the existing law² relating to the nomenclature of fungi with pleomorphic life-cycle. It is an acceptable translation of the text originally adopted for Art. 49 bis (de Wildeman 1910: 63), and as such has been operative since May, 1910. During these thirty-seven years its provisions have been faithfully followed by the majority of mycologists. There have been, however, certain proposals for the modification of its essential principle—or “interpretations” which have the same effect—and it is these modifications which are the subject of the present note.

Although there may have been earlier discussion of the matter, apparently the first formal motion for a change in the Rule was offered by Arthur (1929), and presented by Briquet (1930: 67) for action at the Cambridge congress. This motion provided for three alterations in the Rule then numbered 49 bis: (a) deletion of the reference to the works of Persoon and Fries as starting-points; (b) an addition to the sentence designating perfect states, so as to have it read, “The perfect state is that which ends . . . in the uredospore or the teleutospore (sporophyte) in the Uredinales”; and (c) a complete replacement of the first sentence under “Examples” by the sentences: “The names *Accidium* Pers., *Roestelia* Reb., *Accidiolum* Unger, and *Peridermium* Chev. designate different states of the gametophyte in the group *Uredinales*. The generic name *Accidium* Pers. . . ., belonging to the gametophytic state, cannot displace *Gymnosporangium* Hedw. f. . . ., based upon the sporophyte.” The essence of this last proposed change is to substitute in two places examples proscribing nomenclature based on the aecial³ state for examples proscribing the uredinial.

This motion was discussed by its author and others at a meeting of the subsection of botanical nomenclature at the Cambridge congress; no action was taken (Brooks & Chipp 1931: 598–9). The following day it was announced that Maire and Arthur “were agreed in proposing that Art. 49 bis be maintained until the next

² But cf. footnote 1.

³ The terminology here employed by the writer for rust-spores and -sori is that proposed as standard by Arthur (1905); in quotations the terminology is of course that of the author quoted.

congress" (Brooks & Chipp 1931: 622). There was some further discussion, but no further action, concerning Arthur's motion; by vote of the subsection (Brooks & Chipp 1931: 623) Art. 49 bis was retained and was referred to the editorial committee for review of the wording. The third edition of the International Rules constitutes the report of that committee; as was proper, in view of the vote, no essential alteration was made in the Rule (Briquet et al. 1935: 44, 17). Arthur subsequently declared (1934: 474), "No change in the Rule is advocated. The Rule is to stand as it is." The amendment under discussion was not presented at the following congress (Sprague 1935), and unless it has been renewed in some place that has escaped notice, is not now an issue.

In 1934 Arthur published an "Interpretation of Rule 49 bis." His five pages of discussion, which should be read by anyone interested in the subject, are too long for quotation *in extensu*, and will have to be dealt with in excerpt or summary. The major thesis, as stated in the author's summary, is that "the Rule [quoted at the beginning of this note] excludes aecidiosporic names (of the gametophytic state), but includes both uredosporic and teleutosporic names (of the sporophytic, or 'perfect' state)" (Arthur 1934: 476). This "interpretation," like the motion for amendment which it replaced, would, of course, legitimate much of the nomenclature adopted by its author in his American-Code days. It is supported by an ingenious argument based on the cytological evolution of the rusts:

There are two *states* in every species of this order whatever its generic connection, the gametophytic or haploid, which bears aecidiospores, and the sporophytic or diploid, which bears uredospores and teliospores. . . . In reduced species, especially the so-called short-cycle species, the two states are much curtailed, and some of the spore-forms may be suppressed. . . .

The rule clearly sets forth that "the perfect state is that which ends in the teleutospore." . . . Neither the wording of the rule nor the history of spore development excludes the uredospore, a product of the sporophyte, and a normal part of the "perfect state," although the aecidiospore, a product of the gametophyte, is clearly excluded.

. . . Every permanent generic or specific name must be founded upon the "perfect state," which is equivalent to saying that it must be founded upon the sporophytic state, a state which bears uredospores and teleutospores.

Uredospores . . . are borne on sporophytic mycelium and are binucleate (Arthur 1934: 472-3).

Leaving aside the debatable and much debated implication that dikaryotic cells are diploid and sporophytic, one may summarize Arthur's argument as follows: (a) The uninucleate and the binucleate phases of the life-cycle of rusts are the "states" referred to by the Rules. (b) The "imperfect state" consists of the haploid, or uninucleate, mycelium and the spores that it bears; the "perfect state" consists of the binucleate mycelium and the spores that it bears. (c) Uredospores and teliospores are borne on binucleate mycelium and therefore both belong to the perfect state; pycniospores and aeciospores are borne on uninucleate mycelium and therefore both belong to the imperfect state. (d) Names applied to pycnial or aecial stages are hence only "temporary names," whereas names applied to uredinial and telial stages are, equally, "permanent names."

Whether one is acquainted with the life-cycle of rusts only as exemplified in the discussions of *Puccinia graminis* in elementary textbooks, or has followed the very considerable number of cytological papers that have appeared in the literature beginning with the later 1800's, one must be at a loss to understand the quoted phrases "gametophytic or haploid, which bears aecidiospores," "the aecidiospore, a product of the gametophyte," "aecidiosporic names (of the gametophytic state)," or the further note, "It has been found desirable to exclude aecidiosporic names, or any others applied to the gametophyte . . ." (Arthur 1934: 475). For all aeciospores, like all uredospores and teliospores (exception being made for rare abnormalities), are binucleate, and there appears to be no record of a dikaryotic spore borne on a haploid mycelium. Beginning at least as early as 1896 (Sappin-Trouffy 1896: 77, 219, et passim; fig. 4, 5, 14, 34), aeciospores have regularly been shown to be abstricted from the ends of binucleate hyphae; Arthur's *Plant Rusts* (written with the collaboration of Kern, Orton, Fromme, Jackson, Mains, and Bisby) represents aeciospores as belonging to the "sporophyte or diplophase," along with at least most of the mycelium which bears them (Arthur et al. 1929: fig. 59); Jackson states (1931: 16; cf. also 15-17) that "aeciospores, urediniospores, and teliospores are diploid, and the change from the haploid to the diploid phase takes place ordinarily in the primordium of the initial sorus, regardless of the type of spore to follow." There is

no need to burden the argument with further evidence, which can be cited *ad libitum* and *ad nauseam*.

It is, as earlier stated, difficult to understand such statements as occur, in the works of various uredinologists, assigning aeciospores to the "gametophytic," or haploid, or uninucleate phase. It is true that the aecial primordium is normally formed of uninucleate, or at least non-dikaryotic, cells, and that a part of the cells of the mature aecium may remain so. But the same thing is true, *mutatis mutandis*, of all ascocarps—the fructification is composed of an outer sterile layer of uninucleate mycelium and an enclosed system, of greater or less extent and complexity, of so-called "sporophytic" mycelium, on which are borne the organs of fructification. The asci are not on that account "gametophytic," nor is an ascigerous fructification imperfect. Perhaps the statements referred to, that "the aecidiospore [is] a product of the gametophyte," are to be understood not as literal statements of cytological truth, but as merely conventional, as short-hand attempts to convey that the aecium is usually a mixed fructification—*i.e.*, is usually made up of both mono- and dikaryotic hyphae. The aecium is probably such whether the dikaryotic phase is limited to fusion cells, the short hyphae which develop from them, and aeciospores, as supposed by the earlier workers (*e.g.*, Blackman 1904: 338), or whether there is extensive dikaryotization both within the aecium and of the peripheral mycelium (Allen 1935: 1058–60, and pl. 7, fig. f, h; Brown 1935). There is, however, a not inconsiderable number of rusts in which the telium is likewise a mixed fructification, and others occur where uredinia are made up of both kinds of mycelium (Jackson 1931: 18, 74). Are these telia and uredinia on that account to be called gametophytic? Not many mycologists have thought so. The telia and teliospores of "micro" rusts are quite as perfect, to most students of the fungi, as those of long-cycle species even though they develop at that stage which in long-cycle rusts is occupied by aecia and aeciospores. If development on binucleate mycelium be the criterion of the perfect state, then aeciospores, uredospores, and teliospores are equally perfect, and names based on any of these stages are of equal nomenclatorial standing; if development in a cytologically mixed fructification be the criterion of the imperfect state, then

numbers of rusts forming teliospores are quite as imperfect as those which so far as known form only pycnio- and aeciospores.

So much for the interpretation that "state" means cytological phase. It may be thought that this is a strained "interpretation" of the Rule. It is, nevertheless, one which the text can probably be made to bear. The examples, though not so explicit as could be wished, probably imply a more reasonable meaning. "The names *Aecidium* Pers., *Caeoma* Link, and *Uredo* Pers. designate different states (aecidiosporic with or without pseudoperidium, uredosporic) in the group Uredinales." Not haploid and diploid states, nor uni- and binucleate states, nor gametophytic and sporophytic states, but "aecidiosporic" and "uredosporic" states. So much makes clear one matter: the word "state" in the text of the Rule does not refer to, and is not synonymous with, "cytological phase." Rather it refers either to what is often called a spore-form ("aecidiosporic, uredosporic"), or else, more precisely, to a fructification bearing or containing reproductive structures of a particular sort. Further reading of the examples will confirm this: "*Polythrincium Trifolii* . . . [is] based on the conidial state of [*Phyllachora Trifolii*]" ; "the spermogonial state of species of the genus *Diaporthe*." Of these further examples the first ("conidial state") could refer to either a spore-type or a fructification; but the second ("spermogonial state") can refer only to a fructification: a spermogonium is not a kind of spore. Reading of the rest of the Rule itself, finally, would seem to settle the question: "The perfect state is that which ends in the ascus stage in the *Ascomycetes*, in the basidium in the *Basidiomycetes*, in the teleutospore or its equivalent in the *Uredinales*, and in the spore in the *Ustilaginales*." Since only teliospore and [smut] spore are in any sense spores, while both they and ascus and basidium are reproductive bodies, the one meaning of "state" that makes sense with the whole text of the Rule and with all the examples is "fructification"; and the reference to the rusts must be understood to say, "The perfect *fructification* is that which ends in the teleutospore or its equivalent in the *Uredinales*." ⁴

⁴ Although not provided for by Art. 57, it is quite consistent with that Rule to speak of assimilative states, such as (nonsporiferous) mycelial, or plasmodial.

And finally, the decisive pronouncement of the Rules on this matter is the one already quoted from the authoritative (French) text (see footnote 1, p. 241): "the name *Uredo* is already used to designate an imperfect state." Even though it be admitted that there is a possible confusion between *Uredo* the form genus and a uredo—i.e., the uredinal stage—still it must also be admitted that *Uredo* is rejected by the Rules as an alternative to *Melampsora* not because it is the name of a form genus but because it is used to designate an imperfect state; and it must be admitted further that by inevitable implication the uredo (uncapitalized) is thereby declared to belong to the imperfect state. So far as the Rules are concerned, then, the matter is definitely settled.

The reason for here attempting a solution of the problem raised concerning the uredinal state is not a lively concern for the nomenclature of the Uredinales.⁵ The principle involved extends far beyond that order. Arthur seems not to have been aware of this, for he wrote "Rule 49 bis applies solely to the Ascomycetes and Uredinales" (Arthur 1934: 471). The origin of this misapprehension is not apparent; certainly it cannot be the text of the Article itself, for that begins with the phrase "among Fungi with a pleomorphic life-cycle"—that is, among at least a considerable number of fungi of every class without exception (even the Myxomycetes having had binomials applied to the plasmodial state which except for the operation of this article have the same standing as binomials applied to the sporangial state). Furthermore the Article at least indicates, and probably defines, the perfect state

⁵ Except for the inevitable corollary that it validates the nomenclature applied to aecial fructifications, Arthur's "interpretation" is of perhaps only minor importance for the rusts. In 1930 he stated that "if . . . the uredospore state were not placed on the same footing as the teleutospore state, a great number of specific names would be invalidated and there would result numerous changes"—seventy or eighty out of a thousand species that he had reviewed (Brooks & Chipp 1931: 598-9). By 1934 the situation had bettered itself, for Arthur was able to state, "The only recent work embracing all known species of the Uredinales from every part of the world is that of Sydows' *Monographia Uredinearum*. The work is accurately compiled, and the synonymy is essentially complete. The names of species recognized are with rare exceptions those which are in general use. It is interesting to note that out of 2333 species embraced in this work having teleutospores only 26 species are affected by the varying opinion regarding the uredospore . . ." (Arthur 1934: 474).

not only for Ascomycetes and rusts, but also for the Ustilaginales and [other] Basidiomycetes. It is among the Basidiomycetes not included in the Uredinales that the "interpretation" that the perfect state includes all fructifications of the binucleate mycelium would give most trouble. Arthur was under misapprehension here also, since he wrote, "Uredospores . . . are borne on sporophytic mycelium and are binucleate, a condition which is not generally true of conidia as ordinarily considered. . . . True conidia, as in the Ascomycetes and higher Basidiomycetes, are borne on gametophytic mycelium and are haploid" (1934: 473-4). Now conidia borne on the basidial fructification, and therefore presumably on binucleate mycelium, were described at least as long ago as 1853 (Tulasne 1853: 197, 216-219); they were reported by Patouillard in 1887 (p. 56-67), by Brefeld in 1889 (p. 10; pl. 1, fig. 11), and since then by a considerable number of authors. Lyman, for example, published figures and descriptions of conidia borne on the binucleate mycelium of a number of Basidiomycetes (1907: 168, pl. 20, fig. 52-3; 174, pl. 20, fig. 69; 159; 178; 186, pl. 22, fig. 116-25 and pl. 25, fig. 134). Summaries of conidial forms—including dikaryotic ones—reported in Basidiomycetes have recently been given by Martens & Vandendries (1933: 345-52) and by Biggs (1938: 66-7). A number of these conidial states have of course never been given names, and are therefore of no nomenclatorial importance. The situation is different for the named conidial states.

Monilia candicans Sacc. 1876 is a conidial state recently found by Linder (1942) to be congeneric with the type of *Oidium*, and accordingly redescribed and transferred to that genus. It is the conidial fructification of *Corticium pruinatum* Bres. 1903 (= *Pellicularia pruinata*) (Rogers 1943). Since both conidiophores and basidia arise from the same mycelium, under the concept of a "state" as a cytologic phase Saccardo's epithet "candicans," which long antedates Bresadola's, must replace the latter in either *Corticium* or *Pellicularia*. *Hymenochaete tomentosa* B. & C. 1868 was found by Linder to be no *Hymenochaete*, but similarly a conidial state, and accordingly was renamed *Oidium tomentosum* (Linder 1942); in it also the conidiophores arise from the same repent hyphae which bear the subhymenial hyphae of a *Pellicularia*.

The latter was recently described (Rogers 1943) as *P. lembo-spora*; but if its conidial state is held to belong to the perfect state, the specific epithet must be "tomentosum." *Oidium Morgani* Linder 1942, *O. effusum* (B. & C. 1875) Linder, and *O. pulveraceum* (Ellis 1884) Linder have clamp connections on the conidiophores and therefore could also be considered to belong to the perfect state. At present it would be as difficult to place them in their correct basidiomycetous genera as it would be to decide whether the unconnected uredinial state of a rust should be assigned to *Puccinia* or *Uromyces* or to any of a number of other genera which do not have distinctive uredinial fructifications; but if ever they are connected with a basidial fructification, *O. effusum* and *O. pulveraceum*, because of their early date, might cause nomenclatorial trouble.

Other examples could be cited; but so much for the nomenclature of species. *Rhizoctonia Crocorum*, the type of *Rhizoctonia* Fries 1822, has been shown by Buddin & Wakefield (1927: 125, 134) to be binucleate and to be connected with *Helicobasidium purpureum*, the type of *Helicobasidium* Pat. 1887. Under the interpretation that the binucleate phase is the perfect state, the name *Rhizoctonia* would replace *Helicobasidium*, and a new name would have to be found for the unconnected species now collected in *Rhizoctonia*. *Aegerita candida*, the type of the genus *Aegerita* Pers. ex Fr. 1822, has been shown by Lyman (1907: 167-172) and by von Höhnelt & Litschauer (1907: 810-815) to be connected with a *Peniophora* (*P. candida* Lyman, = *P. Aegerita* H. & L.). The *Aegerita* consists of bulbil-like aggregates of cells, said to fragment into conidia, but when formed connected by well defined clamps. It could then be considered to belong to the perfect stage. If it does, then *Aegerita* is a genus not of the Fungi Imperfecti but of the Basidiomycetes, and must replace *Peniophora* Cooke 1879. One more case: *Michenera artocreas* is the type of the genus *Michenera* B. & C. 1868; it is a cupulate conidial fructification associated with *Aleurodiscus subgiganteus*. Since it sometimes springs from the hymenium of the latter basidiomycete (Lyman 1907: 159), it must be supposed to be binucleate. If all binucleate fructifications are perfect, the name *Michenera* must replace *Aleurodiscus* Rab. ex Cke. 1875.

These examples are taken from the Thelephoraceae not because similar ones could not be found elsewhere, but because that family is most familiar to the writer. The genus *Rhizomorpha* Pers. ex Gray 1821 from the beginning included a number of species, but the most surely recognizable and best known is *R. subcorticalis*, and that evidently must be the type of the genus. That name is based on the familiar shoe-strings (Hartig 1878: 59-62) which develop before and with the sporophores of *Armillaria mellea* (Vahl ex Fr.) Quél. 1872 (or *Armillariella mellea* (Vahl ex Fr.) Karst. 1881). Since the rhizomorphs give rise to the basidiocarps, presumably both structures are dikaryotic, and would belong to the perfect state—in which case the name *Armillaria* (or, if *Agaricus melleus* does not belong to that genus, at any rate *Armillariella*) must be replaced by *Rhizomorpha*. The genus *Rhacophyllus* Berk. & Br. 1871, based on *R. lilacinus*, has agaric-like fructifications bearing instead of gills rows of bulbils (or better, perhaps, peridioles) under the pileus (cf. Petch 1926: 238). The basidiophorous stage is not known, nor even whether the form should be accounted an imperfect agaric or an imperfect gasteromycete. But it has been shown to be dikaryotic, and may be, as suggested by Patouillard (1913: 220), a stage of *Psathyrella disseminata* (Pers. ex Fr.) Gillet 1878, which is *Pseudocoprinus disseminatus* (Pers. ex Fr.) Kühner 1928. If it proved to be so, under Arthur's interpretation *Rhacophyllus* would displace one or both of those generic names.

Faull's discussion (1932: 5-11) of the particular problem of *Milesia* vs. *Milesina*, which Arthur (1933) thought significant in this connection, is actually not pertinent here. Faull's contention is that although the description of *Milesia* applies to only the uredinial stage that name ought to stand against the later *Milesina*, based on the telial stage. But on his showing "some of [White's] type materials [of *Milesia Polygoni* White = *M. Polypodii* White] are teliosporic" (Faull 1932: 8, 23), and therefore White's names are in fact "the earliest which [have] been given . . . to the state containing the form which it has been agreed to call the perfect form, . . . that which ends in . . . the teliospore" (Briquet et al. 1935: 17). The fact that "White's definition of both the genus and the species applied to the uredinial phase only" (Faull 1932: 5) affects

the nomenclatorial status of the genus and species no more than does any other faulty original description—that is, not in the least. The law on this point is contained in Art. 18: “The application of names of taxonomic groups is determined by means of *nomenclatorial types*. . . . The type . . . of a generic name is a species, that of the name of a species . . . is usually a specimen . . .” (Briquet et al. 1935: 3). The opposing doctrine (that an inaccurate description rather than a good specimen determines the character of a species) would mean that most early names of fungi would have to be abandoned—e.g., *Agaricus* Fr., described as “ascigerous” (Fries 1821: 8). *Milesia* is then the valid name—if all of Faull’s statements of fact be accepted as correct—under the Rules.

As earlier noted, Arthur stated in 1934 “that no change in the Rule is advocated.” The agenda for the 1935 congress included, nevertheless, a motion over his name for modification of Art. 57, the former Art. 49 bis (Sprague 1935: 42). It is composed of two parts: the first is to substitute “Linnaeus, *Species Plantarum*” for “Persoon, *Synopsis*.” This is merely a corollary of a motion printed elsewhere to move the starting-point for rusts back from 1801 to 1753, and does not affect the “interpretation” of the provisions concerning pleomorphic fungi. The second part is a proposal to delete all that part of the “Examples” having to do with rusts and to substitute the statement, “The generic names *Aecidium*, *Roestelia*, *Peridermium*, and *Uredo* not only are the names of genera, but are also employed to designate different stages in the group *Uredinales*.” As an “example” this appears somewhat pointless; it is a Rule, if anything; and it certainly is not a mere revision of the sentence it replaces, but a quite different statement on quite a different subject. Whatever the intention of the proposed amendment, its effect is to change the designation of the uredinial and the several aecial fructifications from “states” to “stages.” To that extent it constitutes a change not only in the “Examples” but in Art. 57 itself, and should in the interest of consistency be rejected by those who are not prepared to follow consistently and to its conclusion Arthur’s interpretation of the term “state” and of Art. 57. Even the adoption of the amendment would, however, not constitute unassailable endorsement of that

interpretation, since in the examples taken from the Ascomycetes the state would still be "conidial" or "spermogonial"; but it would render equivocal what is now unequivocal. At the Amsterdam congress (Sirks 1936: 368) the appointment of a special committee to study this amendment was authorized, and presumably the matter will come up at the next congress.

Bisby (1944: 283) has recently offered for discussion a complete revision of Art. 57. His version has much to commend it; it would settle authoritatively a number of points where now one must be guided by inference or custom. It is, however, somewhat less explicit than the existent Rule; in particular, it does not provide any means, as Art. 57 has been shown to do, for determining in what sense "state" can legitimately be used. And like Arthur's proposed amendment, it declares that "the perfect state . . . in Uredinales [is] the Uredo or telial stage." It would seem preferable to insert its provisions concerning type specimens, the citation of authorities, and, if such a provision is desirable, concerning precedence among imperfect states, in their several proper places in the text of the Rules, and to the writer at least, to leave Art. 57 quite, or at any rate in essence, unchanged.

For, as must appear from the present discussion, the "states" referred to by the Rules are not cytological phases, but organs of fructification (or assimilation); and the uredinial state has no greater claim to recognition as a perfect state than has the aecial—or, in various other Basidiomycetes, conidial, sclerotial, and mycelial states. Furthermore, it is illogical (unless Art. 57 is to be abolished utterly), it is contrary to the practise of the great majority of mycologists, and it cannot but be confusing, to treat two or more different states in the same life-cycle as equally perfect, and the names based on them as of equal value in determining the legitimate name of a fungus. Furthermore, the assignment to the uredinial fructification of "perfect" status can apparently be accomplished only on the basis of a principle which would radically and extensively alter the nomenclature not only of the Uredinales (since it would validate names applied to the aecial fructification), but of other and larger groups of Basidiomycetes. And finally, it is proper to take into account that the concept of perfect and imperfect states is not strictly a nomenclatorial one, not even merely

a taxonomic one, but is as well a distinction important in fungus morphology. One who values precision in language, who values language as an instrument for developing and conveying ideas, can scarcely accept with unconcern the debasement of one of the special tools of his trade, the deliberate creation of an ambiguity which renders valueless a single one of the terminological distinctions with which he works.

In this discussion the writer has had the benefit of discussion or comment by Dr. G. R. Bisby, Dr. G. B. Cummins, Dr. B. O. Dodge, Dr. H. S. Jackson, and Dr. G. W. Martin, none of whom, however, is in any degree thereby committed to the conclusions expressed.

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VARIATION IN MONTAGNITES ARENARIUS (DC.)

ELIZABETH EATON MORSE

(WITH 19 FIGURES)

Montagnites is a peculiar fungus which occurs in arid regions in the Western United States. It has been collected on the plains of eastern Oregon, on California deserts, in New Mexico, in the region of San Diego and on the peninsula of California. Its peculiarity lies in the fact that whereas it arises as a two-layered, hypogaeic gasteromycete, in passing through its various stages of development, it approaches at maturity the family Agaricaceae, resembling most the genus *Coprinus*. It has a cap and stem, also a volva such as is met in some coprini (3, p. 206 f. 108). It has on the sides of the gills the large, vesiculose cystidia of *Coprinus*, also black spores. However, it is removed from *Coprinus* by the structure of the gills and by their weak attachment to the cap; furthermore, *Montagnites* never deliquesces.

It thus appears that *Montagnites* represents a transition between two large groups of fungi, gasteromycetes and agarics; it is placed by Clements and Shear in the family Agaricaceae in their *Genera of Fungi*, p. 230, and by Killerman (5, p. 230).

Fries discovered that he and De Candolle were applying the genus name *Montagnea* to two groups of plants far removed, De Candolle (2) to a member of the composite family and Fries to the above fungus. Fries, therefore, decided to change the ending of *Montagnea* just a little ("paullulum"), which resulted in *Montagnites*; he honored his contemporary by appropriating his name for the species name, explaining the situation as follows: "Primo dixi *Montagneam*, sed quum Decandolle eodem anno proposuerit novum genus sub eodem nomine, meum paullulum mutavi." (See Fries, *Hymenomycetes Eur.*, p. 319, 1874.) Fries published *Montagnea* in *Genera Hymenomycetum* (p. 7) in April, 1836; De Candolle published *Montagnea* in October, 1836. Fries' claim to *Montagnea* cannot be disputed, but he decided to use *Montagnites*.

Montagnites candollei was promptly adopted in all the literature, and it seems to me ill advised to try to revive the earlier use of the name *Montagnea* (Mycologia 35, p. 450, 1943). Fries' explanation for its discontinuance should be adequate.¹

It must be admitted that Fries' proposal of *candollei* as a species name is out of order, since two other names had already been given to this fungus—*Agaricus radiosus* Pallas, 1777 (see Hollos, 1904) and *Agaricus arenarius* DC. (see Fries, Epicrisis, p. 241, 1838).

There is extraordinary variation in size, recognized by the French mycologist Patouillard (7, p. 219) in erecting the species *Montagnites tenuis*, characterized also by small spores. Cleland accepts wide variations in spore size in a single species (1, p. 164). Long finds very large and very small specimens growing in close proximity, but advises against even a use of variety.

Hollos (4, 30–33, pl. 1–2) shows in his illustrations numerous rhizomorphs issuing from volvae. We have not observed such in any of the specimens acquired by us. Doubtless they were present but lost in collecting.

Description of *Montagnites arenarius* (DC.) comb. nov.

Agaricus radiosus Pallas Reise 2: 744. pl. 4, f. 3. 1777.

Agaricus arenarius DC. Fl. Franç., tome 5: (vol. 6): 45. 1815.

Montagnites candollei Fr. Epicrisis, p. 241. 1838.

Montagnites pallasii Fr. Epicrisis, p. 241. 1838.

Montagnea candollei Fr. ap. Corda Icon. Fung. 6 (edit. Zobel): 85. pl. 20, f. 146. 1854.

Montagnites radiosus (Pall.) P. Henn. Hedw. Beibl. 40: (98). 1901.

Montagnites radiosus (Pall.) Hollos Gasterom. Ungarns, p. 30. pl. 1, f. 16–23; pl. 2, f. 1–4. 1904.

Montagnea arenaria (DC.) Zeller, Mycologia 35: 418. 1943 (as "*arenarius*").

I can do no better than refer readers to the detailed description of this fungus by Doctor John Burton Cleland in "Toadstools and Mushrooms" (1: 162). A condensed statement made from that description follows:

¹ Conservation of Name *Montagnites*.

In consideration of the almost universal acceptance and endorsement of the name *Montagnites* Fries, I hereby request the next Congress to consider placing it on the list of Nomina Conservanda.

(After Montagne, the French mycologist). Universal veil forming a volva, persistent. Stem dilated at the apex into an orbicular disc smooth on both sides, to the margin of which the free gills are attached. Gills radiating, sickle-shaped, persistent, with obtuse edges, without an enveloping cuticle. Trama cellular. Spores oblong, smooth, black-fuscos, basidia tetrasporous.

Pileus 1.2–2.5 cm., occasionally more, at first deeply inturned below towards the stem, "tucked in," covered by the grayish to dirty white universal veil through which shows the ribbing of the gills. Gills to 3 mm. wide, very close like the leaves of a book, attached along the periphery of the disc, at first covered by the delicate universal veil, surface slightly wrinkled, carbonaceous; on old plants the gills expand outwards and become ragged.

Stem 2.5–7.6 cm. long, 3–7 mm. thick, equal or attenuated upwards, or slightly so downwards.

Cleland records great variation in the shape of spores: "spherical," "irregularly spherical," "elliptical," "triangular," "ovate"; he records spore measurements in very many collections: Mount Elba, $19-24 \times 11-12.8 \mu$ (rarely $27 \times 21 \mu$); Woodford Creek, $15-26 \times 9-15 \mu$; Encounter Bay, (with stem 2 mm. thick) small spores, $7.5 \times 4.4 \mu$, which are elliptical to irregular or almost triangular. It thus seems, "as suggested by others," so states Cleland, "that we are dealing with a single species, highly variable as regards its size and robustness and the size and shape of the spores" (1, p. 164). Lloyd (6, p. 1165) also comments briefly on the extreme variation of this species.

SUMMARY

Greatest stature in Central Europe, Hollós. (28 cm.)

Greatest robustness in New Mexico Coll. Long. Cap $5\frac{1}{2}$ cm. wide, stem to $1\frac{1}{2}$ cm. wide.

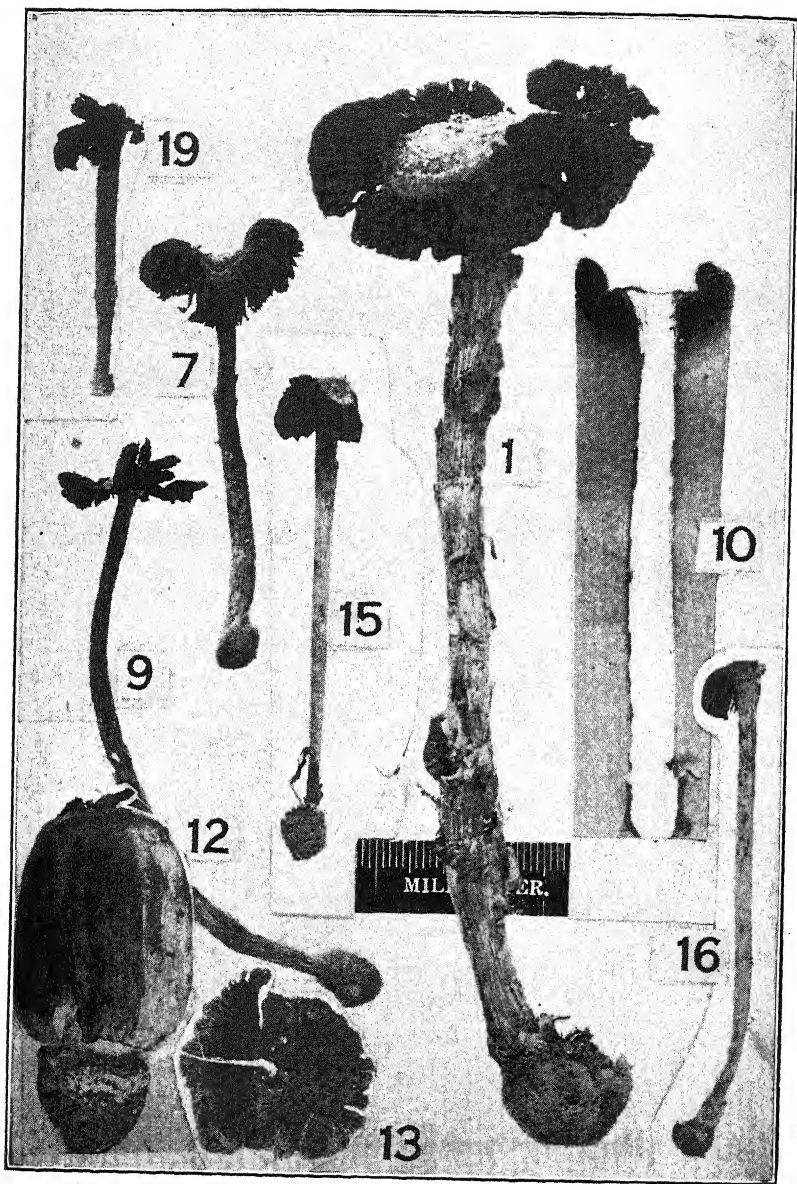
Most diminutive, Galapagos Islands on the equator, Howell.

Darkest scales, Peninsula of Lower California, Mexico, Wiggins and McMurphy.

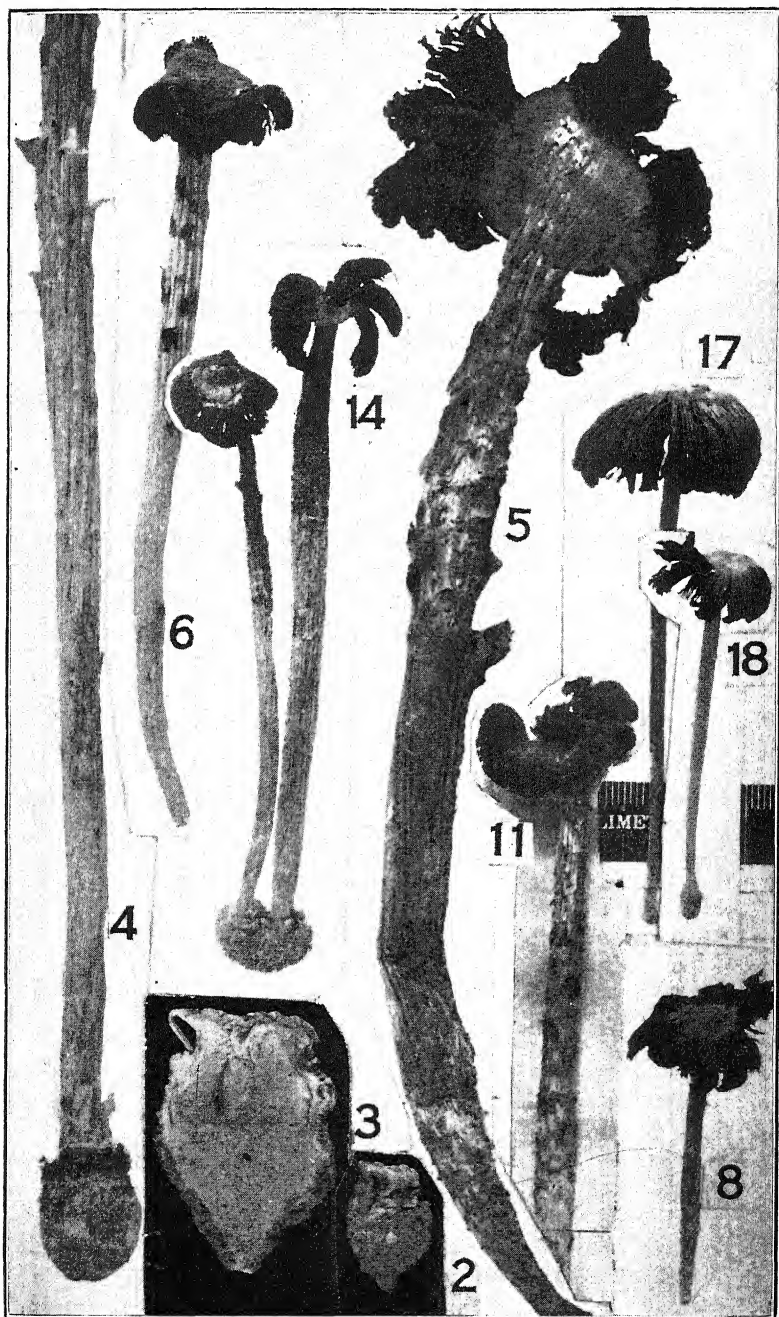
Largest and smallest spores, South Australia, Cleland.

Greatest variability in shape of spores, Central Europe, Hollós.

Narrowest stem, Encounter Bay, 2 mm. thick, Cleland.



Montagnites arenarius.



Montagnites arenarius.

ACKNOWLEDGMENTS

I wish to extend grateful thanks to those who have supplied material making it possible to publish these illustrations; also to my editors and all those who have assisted in many ways.

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EXPLANATIONS AND DESCRIPTIONS

Photographs by W. C. Matthews

- Montagnites arenarius*. FIG. 1. A superior example of a mature specimen, 18 cm. tall, head, stem and volva intact; disc roughened by remnants of universal veil; lamellae crowded, blackened by ripening spores, attached to margin of disc, showing tendency to roll back on the disc; stem equal, cuticle split longitudinally and transversely, broken into coarse scales with tips pointing upwards; volva globose, scaly, sanded, showing ligulate scales issuing at summit of volva; spores $10-12 \times 6-7 \mu$. Dry Lake, near Lancaster, California, Mojave Desert, April 4, 1932. Collector, Harold E. Bailey.
- 2,—Volva, 12 mm. tall, globose, wrinkled in drying, blunt-pointed, sanded, region Albuquerque, New Mexico, June 30, 1937, W. H. Long. ($\times 1$)
 - 3,—Vertical median section, shows 2-layered peridium, spongy basal tissue, plant in embryo discernible. ($\times 2$)
 - 4,—Incomplete stem and volva of a "giant" specimen, woody, deeply ridged, slightly twisted, scales pointing up, orifices often within scales ("chimneys?").
 - 5,—A "giant" specimen, over-mature, stem coarse-scaly, ventricose, widened at summit into a circular disc to the margin of which lamellae, tattered and torn, are attached by a thread, some plates missing; stem narrowed to base, volva lost.
 - 6,—Specimen of medium stature, upper half of stem scaly, volva missing.
 - 7,—A complete specimen, dwarfed.

- 8,—A diminutive specimen, volva lost. FIGS. 2-8. Preserved in U. S. Department of Agriculture, Beltsville, also in the herbarium of the University of California, Berkeley, and in the Long herbarium, Albuquerque, New Mexico.
- 9,—Specimen complete, stem long, slender, curved.
- 10,—Vertical, median section, central tissues somewhat floccose, volva close fitting.
- 11,—Stem, dark-scaled, scales closely adherent, spores $12-16.8 \times 10.8 \mu$. Old, dried specimens from Hamilton Ranch to the Cape, Peninsula Lower California, April 28, 1931, Wiggins and McMurphy. Bank of Salinas River, ranch, Harry Hatch, Templeton, Calif., San Luis Obispo Co., April 2, 1932; another collection, pileus 18 mm. broad, gills extending to 30 mm., making pileus seem that broad; stipe 10-16 cm. tall, 7-8 mm. broad, half buried in sand, Ira L. Wiggins.
- 12,—Var. *texensis* Berk. and Curt., young plant grown in laboratory, with typical volva, remnants of universal veil on top of cap, cap closed down over stipe which elongates rapidly.
- 13,—Underside of a mature cap, broken from hollow stem, showing a weft of tissue, free, floccose. FIGS. 12 and 13, laboratory, Riverside, Calif., Oct. 1939, C. O. Smith. (See Madroño, Vol. 5, No. 4, pp. 119.)
- 14,—Two individuals, each volvate, ensconced in *one* volva, herbarium, Univ. Calif., Berkeley, May 1, 1914, T. S. and K. L. Brandegee.
- 15, 16,—Two slender specimens, volvate, long, ligulate scales issuing from top of volva. 15; collected from arid region eastern Oregon, donated herbarium Univ. Calif., 1944, S. M. Zeller.
- 17, 18, 19,—Small specimens, volvae intact, stems slender, fairly equal, collected off Ecuador, on South Seymour Island, Templeton Crocker Expedition, June 10, 1932, John Thomas Howell; from Miss Alice Eastwood, Academy of Sciences, San Francisco, Calif.

NEW GENERA OF FUNGI-IV

R. SINGER

1. THE GENUS NEOPAXILLUS SING.

A Brazilian agaric collected by J. Rick¹ and now preserved at the Farlow Herbarium exhibits characters that do not fit into any known genus. It is, in certain regards, intermediate between the Cortinariaceae (*Cortinarius*, etc.) and the Paxillaceae (*Lindermycetes*, *Paxillus*), having the spore characters more nearly of a *Cortinarius* (though the ornamentation is not identical with that of any *Cortinarius* known to the author), and the habit and trama of the Paxillaceae. The author considers this collection as the type of a new genus and species.

Neopaxillus Sing. gen. nov.

Habitu *Phylloporum* in mentem revocante; lamellis distantibus, decurrentibus; tramate hymenophorali e mediostrato axillari et hymenopodio irregulariter intertexto consistente, hoc subhymenium circum spatia interlamellaria parallele sequente; sporis globosis, spinulis altis (0.8–1.0 μ) cylindricis obscure ferruginosis obsitis, maiusculis, poro germinativo et callo destitutis; pigmento cuticulae pilei membranali; cuticula palisadam trichodermialem efformante; hyphis omnibus fibuligeris; mycelio albido, sparso, psammigeno; velo apparenter nullo. Species unica, *N. echinosporus* Sing. spec. nov., characteribus generis gaudet.

N. echinosporus has the characters of the genus (see above). The pileus is strongly depressed in the center; it has the size of *Deconica crobula* (or is slightly larger) for which it was mistaken by Rick, but is more fleshy and opaque; its color corresponds to "Spruce yellow" of Maerz & Paul. The terminal members of the trichodermium palisade are broadly clavate, $17\text{--}42 \times 7\text{--}17 \mu$. The lamellae are now a dull rusty color, deeply decurrent, occasionally with anastomosing veins, and often anastomosing at the stipe; the latter is, at least seemingly, evelate, rather thin but not cartilaginous or particularly fragile; the sterile layer of the lamellae consists of

¹ J. Rick, a pioneer-collector of South Brazilian fungi, died recently.

a subhymenium (small cylindric and isodiametric cells immediately underneath the basidia, well developed but not very sharply separated from the hymenopodium), a hymenopodium which is a broad layer of interlaced hyphae, only occasionally some hyphae running subparallel with each other (this layer accompanies the subhymenium all around the bottom of the interlamellar spaces as in the Boletaceae), and the mediostratum which lacks a lateral stratum, at least in the stage seen in the available specimens; the mediostratum proper consists of somewhat wavy, strictly axillary and loosely arranged hyphae; the spores are evidently some sort of brown when seen in mass but a good spore print was not preserved; under the microscope, they are brown-spinose (spines cylindric, $0.8-1.0\ \mu$ long), with a moderately thick wall, without a germ pore or callus, with a moderately large, central oil droplet, and $8.5-10\ \mu$ in diameter; the young, immature spores are smooth, then they become punctate, and finally echinate; the lower part of the spines may perforate part of the wall but this cannot be seen clearly with the methods employed; basidia 2- to 4-spored basidia (the 2-spored basidia sparse to as numerous as the 4-spored ones), $30-48 \times 8.5-10\ \mu$; cystidia were not seen, but there are what may be called cystidioles on the sides of the lamellae, and especially at the edge, versiform, sometimes (on edge) consisting of several isodiametric cells, without contents, $18-50 \times 6.8-8.2\ \mu$. The specimens were collected on sandy soil near Couto, Brazil, in 1936.

2. THE GENUS *MACROMETRULA* DONK & SING.

Massee described a species, obviously introduced to England, as *Agaricus rubriceps* Mass., and lists it in the Friesian subgenus *Chitonina*. The type of this species is preserved at Kew. The author wants to express his gratitude to the Director of the Kew Gardens, England, for loan of this material. Before, however, a systematic attempt had been made on the part of the author to determine into which genus this species actually belongs, Dr. M. A. Donk told the author that he considered Massee's species as the type of a new genus. It was then agreed to propose this genus—if new—under the name *Macrometrula* Donk & Sing. This new genus is—as shown by an extensive anatomical study of the

type by Singer—extremely close to *Psathyrella*, yet still well enough separated by one very striking character, the large, cup-shaped-saccate volva, and by some less important characters. The author has, indeed, hesitated for a while, waiting for more information on the genus *Psathyrella* that might provide a possibility to find transitional forms within the latter genus. The author wants to express his indebtedness to Dr. Alexander H. Smith, the American specialist of *Psathyrella* and related genera, for an interesting discussion on the subject of *Macrometrula* and its relationship with *Psathyrella*. It appears that *Macrometrula* is well enough separated from all groups of *Psathyrella* to justify a generic distinction of the former genus which is characterized by the following diagnosis:

Macrometrula Donk & Sing. gen. nov.

Habitu genus *Volvariellam* (*Volvariæ*) in mentem revocante, sed lamellis adnexis; epicute pilei cellulari *Psathyrellarum* modo, ex elementis isodiametricis dense agglutinatis consistente, subbrunneolis KOH ope in strato inferiore cuticulæ; basidiis magnitudine et forma ea *Psathyrellarum* revocantibus, tetrasporis; cystidiis sursum ventricosis et attenuatis vel subcapitatis ad apicem (typus "*Hygrophilum*"); sporis minutis, poro germinativo lato applanato instructis, duplici et crasse tunicatis, levibus, endosporio hyalino, pallide umbrinis in KOH, fortiter discoloratis ad ardesiacum H_2SO_4 concentrati ope; tramate hymenophorali pallide brunneolo, subregulari, cum sporis non-amyloideo; subhymenio densissimo, ex elementis minutissimis efformato; stipite centrali, cavo, exannulato, e volva bene evoluta, basali, firma, membranacea, saccata oriente; carne alba, ex hyphis fibuligeris consistente. Ad terram in calidariis. Typus generis: *M. rubriceps* (Cooke & Mass.) Donk & Sing. comb. nov. (*Agaricus rubriceps* Cooke & Mass.).

FARLOW HERBARIUM,
HARVARD UNIVERSITY,
CAMBRIDGE, MASS.

NOTES AND BRIEF ARTICLES

BOOK REVIEW

SMITH, ALEXANDER H., *North American Species of Mycena*, pp. i-xviii + 521. *pl.* 1-99. *text figs.* 1-56. University of Michigan Studies, Scientific Series, Vol. XVII. University of Michigan Press, Ann Arbor. 1947.

It is a pleasure to read a taxonomic work as well written as this comprehensive treatise on *Mycena*. The reader at once realizes that this is not merely another book on a genus of the fungi, but it represents Smith's own personal contacts with most of the 237 accepted species presented.

This work on *Mycena* has followed to a wise degree the modern trend toward natural divisions on morphological characters as opposed to artificial groupings which have been especially the vogue in previous classifications within the Agaricaceae. Smith says, "Closeness of relationship, which is what we are dealing with (within genera), is based on degree of similarity in characters accepted as fundamental. Since the taxonomist is working almost entirely from circumstantial evidence, there is not much to be gained by insisting that a classification be based on relationship when the point has been reached where affinities are difficult to ascertain and must be determined on secondary characters. Consequently, in my estimation, the most satisfactory arrangement of species will always involve some compromise between artificiality and naturalness."

In the segregation of genera somewhat similar to *Mycena* emphasis has been rightly placed on relationships and a broader rather than a narrower concept than that of Fries has been adopted. "It is evident that in this genus speciation has proceeded largely on microscopic characters, such as those of spores and cystidia, and because of this, I have not seen fit to use these at the generic level. By adopting a broad concept, the relationships of the species, particularly those which formerly were regarded as borderline species between *Mycena* and *Collybia* and *Omphalia*, could be more satisfactorily expressed. At the same time, *Mycena*, as a concept, is

maintained for a group of fungi so similar in general appearance that anyone with a small amount of experience can nearly always recognize it at sight in the field. To me, this is one of the criteria of a truly natural genus."

This comprehensive treatment of *Mycena* in North America satisfies a long-felt need by those who have in any measure attempted its study. Atkinson and Kauffman had started monographs of the genus, utilizing microscopic characters to delimit species, and Smith has expressed warm appreciation of the stimulus Kauffman personally gave through frequent discussions and Kauffman's wealth of knowledge of the genus gained by years of experience.

A concise but accurate history of *Mycena* in North America is included, giving recognition to the contributions of Schweinitz (1822-1834), Curtis (1867), Peck (1872), Harkness and Moore (1881), Murrill (1916), Kauffman (1918), and Beardsley and Coker (1924).

For the most part, American mycologists have saved type specimens of new species, making possible studies of microscopic details, and thus avoiding "the confusion of concepts that has characterized European agaricology since the time of Fries. However, since many American students did not pay close attention to microscopic details, numerous misidentifications have appeared in our literature, and a multiplication of names for some species has resulted."

Smith's description of *Mycena* delimits the genus quite precisely, as Kühner has done. "Although the arrangement presented here differs in some particulars from that of Kühner, it is in close agreement with his." Kühner recognized 143 species of *Mycena* in Europe whereas Smith accounts for 218 from the United States and Canada and 19 from tropical North America. The author regrets that due to the war, he was unable to make a comparative study of collections of *Mycena* in European herbaria.

The genus is divided into the four subgenera, *Pseudomycena*, *Eumycena*, *Glutinipes*, and *Mycenella*. These are subdivided into sections (and subsections in *Eumycena*) which are often again divided into stirpes. *Pseudomycena* includes two sections containing six species, *Eumycena*, 11 sections and numerous subsections containing 185 species, *Glutinipes*, four sections containing 21

species, and *Mycenella* has two sections containing six species. The section typical of *Eumycena* naturally forms the hub about which the other sections and subgenera are grouped, and contains the type species, *M. galericulata* (Fr.) S. F. Gray. The keys to the subgenera and sections are for the most part based both on macroscopic and microscopic morphological characters.

The author devotes a chapter of more than 20 very interesting pages to an evaluation of the various diagnostic characters used by him in speciation. Consideration is given to seasonal occurrence, habit and habitat, size and stature of fructifications, color, macroscopic characters of the surface of both pileus and stipe, lamellae, flesh, latex, macroscopic characters of dried specimens, and such microscopic characters as spores, basidia, cystidia, structure of gills and pileus, iodine reaction of the flesh, and viscosity of the stipe.

The author directs our attention to Kühnér's¹ excellent and more extensive account of the anatomical details of the various types of carpophores grouped in *Mycena*, since the present work considers only characters emphasized by Smith in his arrangement and description of species.

There is included a short chapter on the technique of (1) collecting and drying specimens, (2) preparation and study of fresh and dried material, and (3) chemical tests.

The following 29 new species and seven new varieties are described: *Mycena albicolor*, *M. albissima*, *M. arenaria*, *M. Brownii*, *M. cayugaensis*, *M. cheboyganensis*, *M. cineraria*, *M. cylindrospora*, *M. filiformis*, *M. fuliginella*, *M. Gaultheri*, *M. griseoviridis*, *M. incarnatifolia*, *M. kalalochensis*, *M. Kauffmaniana*, *M. Kuehneriana*, *M. litoralis*, *M. paucilamellata*, *M. pseudoclavicularis*, *M. pseudogrisella*, *M. pseudoinclinata*, *M. Rickeni*, *M. setulosa*, *M. subcana*, *M. subconcolor*, *M. subfusca*, *M. tenuiceps*, *M. thujina*, and *M. umbrina*; *M. alnicola* var. *odora*, *M. epipterygia* var. *lignicola*, *M. flavoalba* var. *microspora*, *M. griseoviridis* var. *cascadensis*, *M. olida* var. *americana*, and *plumbea* var. *robusta*. There are three species newly named, i.e., *Mycena Atkinsoniana* (*M. fagicola* A. H. Smith, not *M. fagicola* Grog.), *M. rubrotincta* (*M. tenuicula*

¹ Kühnér, Robert. Le Genre *Mycena* (Fries). Encyc. Myc. 10: 1-710. 1938.

Murr., not *M. tenuicula* Karsten), and *M. subvestita* (*Omphalia vestita*, not *M. vestita* Velenovsky).

Among the excluded species is the new combination *Hygrophorus acutoconicus* (Clements) Smith (Syn. *Mycena acuto-conica* Clements), and the following 16 new combinations resulted from transfers from *Omphalia* or *Omphalopsis*: *Mycena albidula* (Pk.), *M. bisphaerigera* (Lange), *M. delicatella* (Pk.), *M. lilacifolia* (Pk.), *M. McMurphyi* (Murr.), *M. misera* (Fr.), *M. pallida* (Murr.), *M. papillata* (Pk.), *M. pseudogrisea* (Murr.), *M. pusillissima* (Pk.), *M. semivestipes* (Pk.), *M. serotina* (Pk.), *M. subimmaculata* (Murr.), *M. Swartzii* (Fr.), *M. translucentipes* (Murr.), and *M. turbinata* (Murr.).

The author is to be commended on the consistently good photographs well reproduced in 99 half-tone plates which illustrate in natural size 130 species and varieties.

There are also 56 full pages of text figures containing line drawings of the cystidia and spores of more than 200 species and varieties of *Mycena*.—S. M. ZELLER.

NOTICE OF FORAY

The Annual Foray will be held June 15–17 at the University of Michigan Biological Station on Douglas Lake, Cheboygan Co., Mich. The early date, necessary because of the September meetings of the A.A.A.S. and the Mycological Society, will give members an opportunity to see and collect a somewhat different flora from that found in early autumn. The region is rich in bogs as well as hardwood and coniferous associations. Dr. A. H. Smith, who has collected in the area in early summer for several seasons, states that such forms as *Underwoodia columnaris*, *Rhodotus subpalmatus*, *Otidella fulgens*, *Collybia tenuipes*, *Mitrula phalloides*, three species of *Crinipellis*, large numbers of smaller discomycetes, species of *Morchella* and *Helvella* and many species of *Mycena*, *Psathyrella* and *Inocybe* should be available. Laboratory facilities will be furnished by the station. Board and room will be approximately \$3.00 a day per person. Communicate by June 10 with F. K. Sparrow, Botany Dept., University of Michigan, Ann Arbor, if you plan to attend. Give details of number of persons and length of stay.—F. K. SPARROW.

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XL

MAY-JUNE, 1948

No. 3

THE DEVELOPMENTAL PATTERN WITHIN THE GENUS PLEOSPORA RAB.

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(WITH 56 FIGURES)

The taxonomic situation in the genus *Pleospora* is similar to that to be found in many other large genera of the fungi, or for that matter any group of organisms which, as a result of a successful adaptation to their habitat, is in an active state of evolution. In a previous study (5) of this genus in northwestern Wyoming, it was pointed out that if a sufficiently large number of collections were examined and tabulated according to some important character such as spore size, there would be found a long series of collections with overlapping variations, within which it would be impossible to detect any natural specific lines of distinction. Since this earlier study loans kindly made by a number of herbaria have made it possible to study some 500 collections of *Pleospora*, which have given a far more extensive, though yet far from complete, picture of the natural population of this genus.

The same general situation still obtains. Although occasional collections or groups of collections stand out as distinct, the great majority fall into larger groupings showing the overlapping variations mentioned for any one of a number of important characters. Although an analysis of these variable characters shows that they vary independently of one another, yet there are certain general

* Papers from the Department of Botany, University of Michigan, No. 864.

[MYCOLOGIA for March-April (40: 127-268) was issued May 4, 1948]

correlations which enable one to plot the general pattern of evolutionary tendencies within the natural population.

Once this pattern is determined, all collections can be arranged in series accordingly and the range of variation in nature more nearly determined. The circumscription of species, following such a policy, is in the main the segregation of sections of these series or species-complexes upon the basis of a loose correlation of the factor variants or often upon a purely arbitrary basis. It soon becomes evident that in either case there are bound to be intermediates or modifications, so that extremes of any one species will approach or overlap those of neighboring ones.

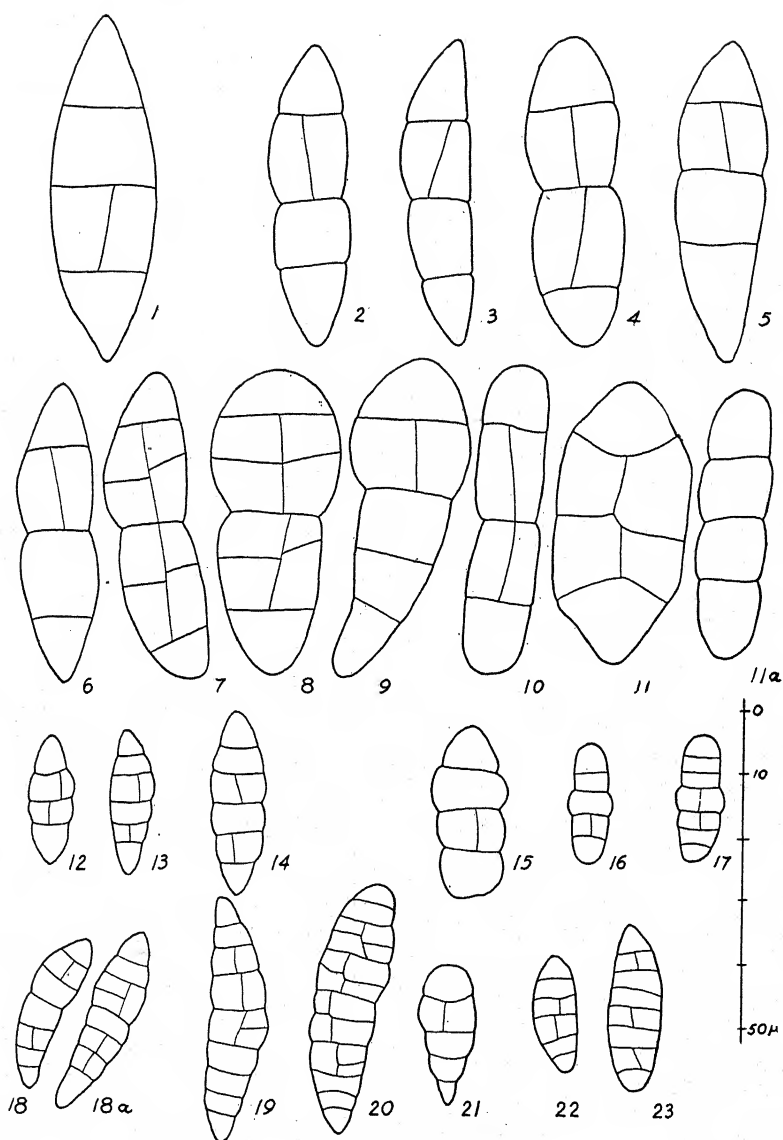
In the past, most species within the genus have been described upon the basis of one or at most a few collections. Such collections at the two extremes of any series or species-complex appear quite distinct. As other collections intermediate within the same series are found to differ in certain factors, they are again described as new. Such a backward approach to the problem ultimately leads to a confusion of overlapping species, which necessitates the description of nearly every collection as a new species.

It is not the purpose of the present paper to delimit species nor to assign binomials, but rather to analyze the variable factors which are of diagnostic importance and to plot the developmental pattern within the genus.

ASCOSPORES. As might be expected, the characters of the ascospore, its form, size, septation, and color are the most useful in determining relationships.

Form. The muriform spore of *Pleospora* has no doubt been derived from that of *Leptosphaeria* by the insertion of vertical septa in some of the cells. The basic simplest spore-form is the fusoid, three-septate, straight, symmetric, non-constricted one shown in figure 1. An analysis of the many spore forms within the genus shows that they are the result of various combinations of the following four modifications of this basic form. The descriptive terms given are those used for these modifications.

1. An increase in the area of the side walls of the spore after the laying down of the transverse septa results in constrictions at the septa. Such constrictions are usually greatest at the first septum

FIGS. 1-23. Spores of *Pleospora*.

to be formed (usually the central one). *a.* Non-constricted. *b.* Constricted (FIG. 2).

2. An unequal development of the side walls of the spore, so that one side becomes flattened or concave, whereas the opposite side remains convex. *a.* Equilateral. *b.* Inequilateral (or curved) (FIG. 3).

3. A rounding of the end walls of the spore, resulting in an ellipsoid or oblong shape. *a.* Ends acute. *b.* Ends rounded (or abruptly tapered) (FIG. 4).

4. An unequal development of the two halves of the spore, on opposite sides of the primary septum, resulting in an asymmetric spore. In such cases the upper portion is usually shorter, broader, and commonly more rounded, whereas the lower portion is longer, narrower, and often tapered. *a.* Symmetric. *b.* Asymmetric (FIG. 5).

A fifth spore form is the clathrate type (FIGS. 11 and 11a), in which the spore is flattened and vertical septa are formed in only one plane, so that they show in the face, but not edge view of the spore. These species have been placed in a separate genus *Clathrospora*, and do seem to form a distinct group.

Combinations of the above factors give the six main spore forms found in the genus and referred to as follows: The *fusoid* type (FIG. 6) characteristic of the leptosphaeroid series. The *fusoid-ellipsoid* type (FIG. 7) characteristic of the *vulgaris* series. The *oblong-ellipsoid* type (FIG. 8) characteristic of the *herbarum* series. The *clavate* type (FIG. 9) often associated with asymmetrically septate spores. The *oblong* type (FIG. 10) found in only a few species. The *flattened clathrate* type (FIGS. 11 and 11a) found in *Clathrospora*.

Septation: The basic spore of the genus is three-septate. The central septum is usually laid down first, but soon is followed by two secondary transverse septa, one on each side. Beyond this three-septate condition there are several modes of septation, as follows.

In the leptosphaeroid series the transverse septa are laid down first and secondary septa are then formed in the end cells giving a five-septate and then a seven-septate spore. This septation of the end cells often leaves the central cells somewhat larger, and one or

both of them may become somewhat swollen, as is commonly the case in *Leptosphaeria*. In this series the vertical septa are rather tardily formed and many, or even all, of the cells may be without them.

In the *vulgaris* and *herbarum* series the secondary septa first appear in the central cells and the vertical septa are usually formed first. In such a vertically septate central cell the transverse septa formed on each side of this vertical septum are often laid down at different times, at different levels, or at different angles (FIGS. 7-8), giving them a characteristic irregular arrangement. The seven-septate spore arises by septation of the two end cells, which is also commonly irregular in that the vertical wall splits into a "Y" shaped septum or shows a transverse septum on only one side. Further septation is variable and will be discussed under these series.

The asymmetric type of septation results from the insertion of extra transverse walls, nearly always in the lower end of the spore. This type of septation occurs in all types of spores and does not constitute a separate series.

Color: The color of the spore in *Pleospora* varies from a pale honey yellow through darker yellow-brown to a dark red-brown. This of course ignores the hyaline spores of *Catharinia*, *Pleosphaerulina*, etc. The simpler leptosphaeroid spores are nearly all yellow-brown. The red-brown color appears to be more or less correlated with growth at higher altitudes or latitudes. Many species show this complete color range, unless color itself is used for species separation. The color also darkens with maturity, and a single collection may show a wide range of color.

PERITHECIA. The perithecium of *Pleospora* has a dothideaceous (pseudosphaeriaceous) type of development. Consequently the cavity arises as a differentiated central area within a stroma, and the wall is in reality the remainder of the sterile stromatic tissue. Great emphasis has been placed in the past upon the comparative thickness of this wall, and such genera as *Scleroplea* have been based upon it. Certain species, as *P. herbarum*, are characterized by a thick wall, but this thickness depends upon environmental conditions of growth and is only of secondary importance even for species differentiation. The same applies to perithecial size which

may vary greatly within one species under varying conditions, but in other cases, in correlation with other characters, may indicate distinct groupings.

The formation of setae about the ostiole of the perithecium has also been used for generic separation (*Pyrenophora*). Many species of *Pleospora* will show a few, brown, creeping hyphae growing out from the perithecium, particularly at the base. All degrees of tomentosity from this to a dense outgrowth of basal tortuous brown hyphae or a woolly mass of hyphae covering the entire perithecium can be found. In these latter cases there is a tendency for the hyphae on the surface of the perithecium to become stiff, upright and pointed, and to penetrate the overlying epidermis. At other times these upright setae are numerous and definite. This tendency for the formation of setae is also more pronounced at high altitudes and latitudes, and also where the overlying host tissues are fragile or soon thrown off. If arranged according to spore form, septation, size, and color, these setose forms comprise a series almost directly parallel with forms having smooth perithecia. The setose condition seems to be of no more than specific or even varietal or habitat rank. A similar opinion has recently been put forward by Petrak (2, p. 446).

ASCI. The asci of the genus vary in shape from almost globose or saccate, through broadly clavate to cylindric. They nearly all have a thickened inner gelatinous wall, often more pronounced above, and an expanded, disc-like or "claw-like," basal attachment. They vary in size and shape with maturity, becoming narrower and longer as the spores are ejected. Although often helpful as a secondary character, they are not of prime importance in the delimitation of species.

HABITAT. There are several modifications which seem to be correlated with habitat. Collections on leaves, petioles, sepals, or minute stems often show small perithecia (200 μ or less in diameter), but similar small perithecia may also occur on herbaceous stems, and larger perithecia on leaves. This appears to be an effect of the habitat rather than an adapted variation of the fungus.

A more intriguing habitat group, but one more difficult to fix, is the one on woody stems. On wood and bark the perithecia tend to be larger with thick stromatic walls and often clustered or con-

fluent, and the asci are more numerous, elongate, cylindric and with uniseriate spores. Although these characters are not definitely correlated with this habitat, most species with a wide variety of spore size, form, and septation show one or more of these characters. These characters are also found in the genus *Teichospora*, which is probably derived from this group.

All of the characters discussed may vary independently of one another, but certain characters or combinations often persist through a series of changes in other characters. The important question is how much variation may occur within an individual mycelium or a species. If one represents the four contrasting pairs of characters of spore form by the indicated letters *a* and *b*, the number of septa by a numeral and the color by Y (yellow), B (brown) or R (red-brown), a formula can be written for any given spore. Such a spore as in figure 1, for instance, would be *a,a,a,a,3,Y*, or for figure 8 perhaps *b,a,b,b,5,B*. With such formulae as a mechanical aid, it soon becomes apparent that the spores of any collection, any perithecium, or even a single ascus, cannot all be represented by the same formula. Different collections, apparently of the same species, will show different percentages of spores having certain formulae. Again the factors of form, color, and septation change with maturity of the spore. In any mature collection, however, the spores do not go beyond a certain stage of development. The most mature spores, therefore, are the diagnostic ones.

Theoretically, an individual is the mycelium, perithecia, conidia, and ascospores from a single spore. In nature, however, such a derivation is next to impossible to determine. Most infections are probably from more than one ascospore or conidium, and it is a common observation to find several species of *Pleospora* mixed upon one stem or leaf. Even in the comparison of spores of a single perithecium or ascus, the possibility of heterokaryosis or hybridization must be taken into consideration. Until the genetic mechanism of perithecial and ascus formation in the genus is known and genetical studies have been made, therefore, the behavior of these factors cannot be known and specific variation and segregation must be based upon personal judgment from morphologic evidence.

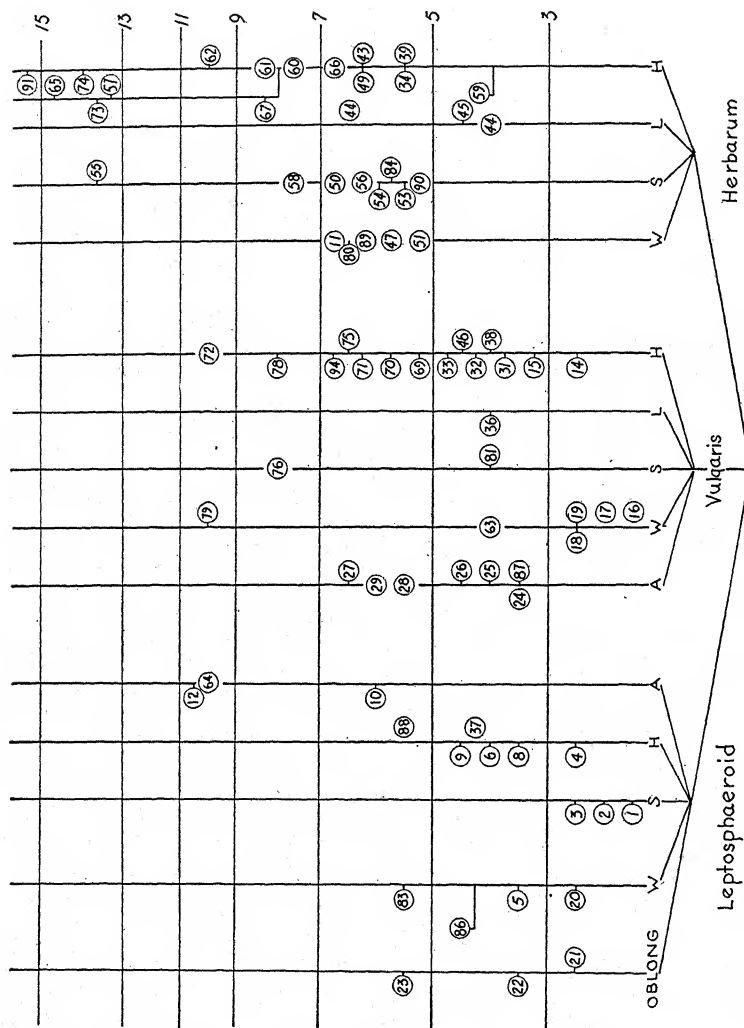


CHART I. A diagrammatic representation of the developmental pattern within the genus *Pleospora*.
Explanation in text.

Chart I is an attempt to plot the natural arrangement of the collections studied. The three main branches of the chart represent the three main series of development within the genus, in each of which a typical spore form is retained. These are indicated as the *leptosphaeroid*, *vulgaris* and *herbarum* series respectively. In each of these series certain groups of species are related upon the basis of other characters. These are represented as secondary branches and designated by letters as follows:

- H. The typical forms on herbaceous stems.
- L. Leaf inhabiting forms which sometimes are distinct enough to represent species, but in others are not.
- W. The wood inhabiting forms which in most cases show the distinctions already enumerated.
- S. Those collections with setose (not merely tomentose) perithecia, which usually run parallel with and correspond to species with non-setose perithecia.
- A. The collections showing spores with asymmetrical septation appear to have been derived from various types with a symmetric septation, and do not form a distinct series.

The small group with oblong spores, at the extreme left, seem to be related to the *leptosphaeroid* series. The horizontal lines represent levels of septation, the number of septa at each level being indicated at the right. The numbers enclosed in circles indicate what seem to the writer to be natural species-groups. Inasmuch as these groups of collections do not always coincide to species as now described, or include several described species, they are referred to here by number only. When such species groups have spores which are predominantly yellow-brown, the encircled numbers are placed to the left of the vertical line; if the spores be red-brown, to the right of the line; or if the spores range from yellow to red-brown, the encircled numbers are placed upon the line.

LEPTOSPHEROID SERIES

This series is derived from the basic fusoid, three-septate, yellow-brown spore. Throughout the series the spores are recognizable in having the following general characters: 1. They are mostly nar-

rowly fusoid, with gradually tapering, acute ends. 2. They are more symmetrical, equilateral and less strongly constricted than in the other two series. 3. The color is always yellow-brown, and often pale yellow-brown. 4. The septation is distinctive and of the type previously described for this series.

Figures 12 to 20, which are spores of this series, are illustrative of these characters. These figures of spores, and the remaining ones (except for the first 11 figures), are camera lucida drawings made to scale and chosen as typical of each particular collection.

The spore in figure 12 represents species No. 4 of the chart, which has three-septate spores in smooth perithecia commonly found on *Papaver*, and is probably *P. pellita* (Fr.) Rab. and *P. papaveracea* (Not.) Sacc. Similar spores found in tomentose perithecia with some setae probably belong to *P. calvescens* (Fr.) Tul. (species No. 3). *Pyrenophora hyphasmatis* E. & E. (No. 1), with tomentose perithecia, and *Pleospora delicatula* Vestergren (No. 2), with setose perithecia, both have even simpler three-septate spores which often entirely lack vertical walls in the cells. The insertion of two secondary septa in the end cells gives the five-septate spore of this series as shown in figure 13, taken from an unidentified collection on *Phleum* from Sweden, and is characteristic of species No. 6, which has small perithecia, is commonly found on grasses, and probably corresponds to *P. vagans* Niesl. The spores of this group range from 18 to 30 μ in length, the largest spores being found in type material of *P. fuegiana* Speg. and *P. Forsteri* Speg. from Tierra del Fuego. Figure 14 is such a large spore found on the type of *Leptosphaeria Yerbæ* Speg. It is not the *Leptosphaeria* described by Spegazzini, but a *Pleospora* of the *vagans* type but with large spores, large perithecia, and on woody stems (No. 83). Other woody forms found in this series are No. 20, a collection of *P. Thuemeniana* Sacc. on *Agave*, with three-septate spores, and No. 5, a collection of *P. parvula* Berl., on *Clematis* with 3-5-septate spores. No. 86 (FIG. 21) is an undetermined collection on *Sorbus* from Sweden having asymmetrically septate spores with four to six septa.

This tendency toward asymmetric septation by the formation of extra septa in the lower end cell of the spore also occurs in herbaceous forms. No. 10 (FIGS. 18, 18a) includes type material of *P.*

intermedia Speg., and *P. Niessleana* Kze., and collections of *P. dura* Niessl which have spores with six to eight septa. No. 12 (FIG. 19) is a collection of *P. dura* which shows a further insertion of septa to give spores which have eight to ten septa.

No. 64 (FIG. 20) is an example of one of the troublesome intermediate forms. It is a collection of *P. abscondita* Sacc. & Roum. on *Phragmites* and shows asymmetrically septate spores with three to four septa above and four to five, rarely six, below the primary septum and therefore is eight to ten, rarely eleven septate. Furthermore there are secondary septa often laid down in the manner described for the *vulgaris* series. The figure shows an extreme case of this. Such septation is also rarely found in Nos. 10 and 12 (FIGS. 18a, 19). In other words, as the number of septa increases in the leptosphaeroid and *vulgaris* series, the spores approach one another in appearance and the two series are difficult to distinguish.

Nos. 37 and 88 are also rather anomalous species, both occurring on *Galium*. No. 37 (FIG. 22) is an unpublished species, labeled *Pleospora Galii* Romell in the Riksmuseum Herbarium. The spores are five-septate and have the general fusoid-ellipsoid form of the *vulgaris* series. The spores are not constricted, however, and show the leptosphaeroid transverse septation, with vertical septa in only a few central cells. No. 88 (FIG. 23) is an undetermined collection, also in the Riksmuseum Herbarium. The spores are identical in form, but larger and with seven septa. Such intermediate species as these merely demonstrate that the "series" as here outlined are merely generalizations covering the majority of cases, and that other combinations of characters may, and do, exist.

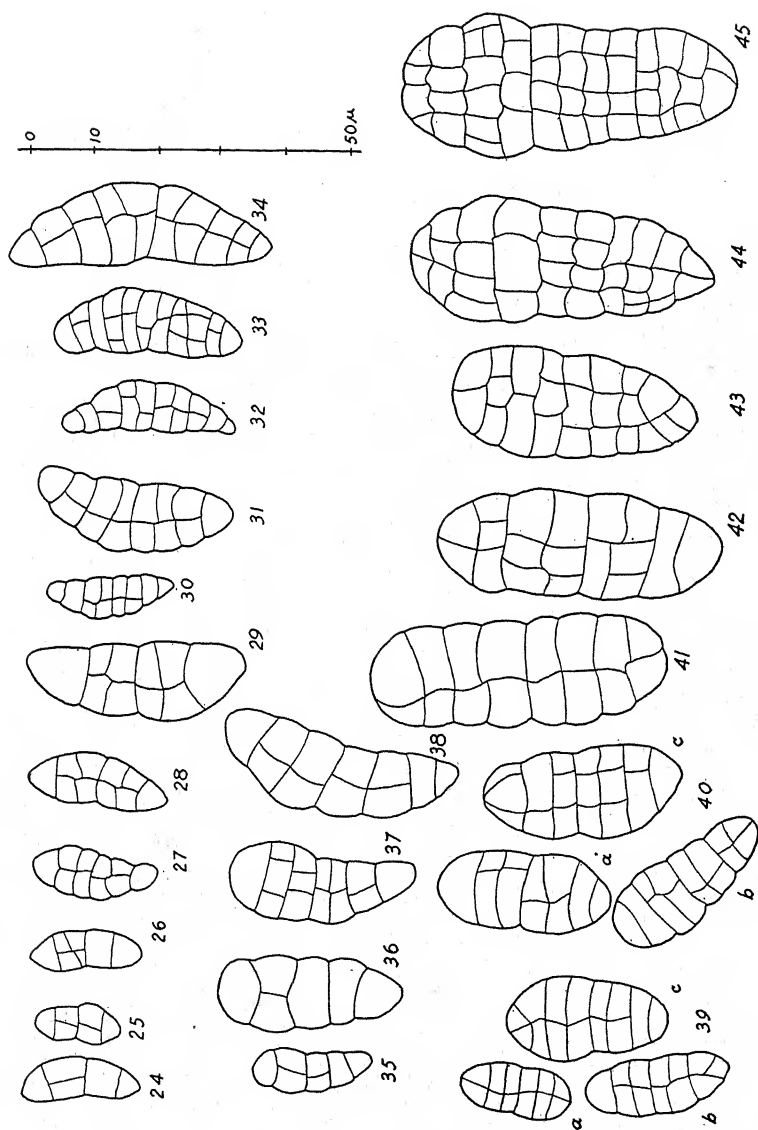
Nos. 15, 16, and 17 are again a small group of species with an oblong spore-form, which are represented as a subdivision of the leptosphaeroid series because of their septation. No. 21 (FIG. 15) is represented by the type of *P. mollis* Starb. These spores are dark red-brown, which is atypical for this series. No. 22 (FIG. 16) has spores with four or five septa (an extra septum in the upper half in the latter case) and agrees with the description and collections of *P. oblongata* Niessl. No. 23 (FIG. 17) has the same spore

form but with six septa. Two collections by Rehm (labeled as a variety) in Riksmuseet show these spores.

VULGARIS SERIES

This series is based upon a somewhat subtle change in spore form, and a type of septation differing from that of the preceding series as already described. The spores have more bluntly tapered or rounded ends, are more commonly inequilateral or curved, and the end cells are never septate. Figures 24 through 38 show spores of this series. It can be derived from the three-septate level, through collections such as are found in No. 14 (FIGS. 24, 25), which show a broadening of the spores and rounding or blunt tapering of the ends. No. 14 includes the type collections of *P. oligostachyae* E. & E. (FIG. 24) and *P. diaporthoides* E. & E. and others, in which the spore form is somewhat variable but always with blunted ends. Some collections of *P. diaporthoides* show some spores with secondary septa of the *vulgaris* type in the central cells. This condition is more commonly found in type material of *P. Boldoae* Speg. (FIG. 26), which is intermediate between No. 14 and No. 33 but is placed in the latter group.

The five-septate condition is attained in this series by the vertical and transverse septation of the two central cells. This stage is represented by Nos. 31, 32, 33 and 38 which comprise a large number of collections with three- to five-septate spores showing many overlapping variations in spore form and size ($14-26 \times 5.5-9 \mu$). No. 31 represents *P. socialis* which has rather small spores and perithecia formed in heavily blackened areas. No. 32 is *P. infectoria* Fck. the type of which shows similar spores (FIG. 27) in perithecia clustered in linear groups on grass stems. The remaining collections of this sort are placed in No. 33 (FIG. 28) which probably corresponds to *P. vulgaris* Niessl. The three species groups just mentioned all have yellow-brown spores. Collections of this type from western America show slightly larger spores with a dark red-brown color and have been considered a variety (No. 38) of *P. vulgaris*. In this same region, collections with these red-brown spores show a continuation of the range of spore size far beyond that of *P. vulgaris*, reaching $26-35(40) \times 8-14 \mu$. This larger spore group corresponds to *P. richtophensis* E. & E. (FIG. 29).



FIGS. 24-45. Spores of *Pleospora*.

The next step is the insertion of a transverse septum in each end cell to form a seven-septate spore. In such cases the inner cell so formed may become vertically septate but the terminal cell remains without a vertical septum. Spores of this type are found in a collection labeled *P. rubicunda* Niessl (but not that species), in Riksmuseet (No. 69, FIG. 30) and of *P. Thurgoviana* Wegelin (No. 70, FIG. 31). Nos. 70, 71 and 94 are all very similar and occur on Monocotyledons such as grasses, rushes, *Typha*, etc., inhabiting swampy margins. They differ chiefly in their perithecial configuration. No. 75, *P. tomentosa* Wehm., has similar but dark red-brown spores and densely tomentose perithecia.

A nine-septate condition is reached in this series by the insertion of an additional septum in the end cells. This is shown in spores (FIG. 32) from the type of *P. Alismatis* E. & E. (No. 78), which may be considered as a continuation of the series mentioned above on aquatic Monocotyledons.

In another group of collections found under *P. Anthyllidis* var. *Aconiti* Rehm (FIG. 33), *P. rubicunda* Niessl, etc. (No. 72), and probably belonging to the latter species, the spores reach an eleven-septate condition with five septa on each side of the primary one. These spores are broader, with more rounded ends, and have one to three vertical septa in the central cells. Here four of the central cells may show the *vulgaris* type of septation.

It will be noted that the irregular "*vulgaris* type" of septation occurs only in the two central cells of spores with seven to nine septa, leaving the two or three end cells with a "leptosphaeroid type" of septation. It was also pointed out under the leptosphaeroid series that the asymmetric types with nine to eleven septa might show the *vulgaris* septation in some cells. This makes it difficult to separate these two series, but since nearly all the spores with more than seven septa are rather bluntly rounded and inequilateral or curved, they are placed in the *vulgaris* series.

Although many collections of the *P. vulgaris* type have perithecia which are tomentose, very few have been seen with true setae. No. 81 has spores of the *P. richtophensis* type, but lighter yellow-brown, and setose perithecia, and is an undetermined collection. *P. pleosphaeroides* Wehm. (No. 76, FIG. 34) has nine-septate

spores, as in *P. Alismatis*, but much larger, and perithecia covered with stiff spines.

The woody forms are also rather restricted in this series. The type of *P. atromaculans* Rehm (No. 16), on *Cornus*, has small three-septate spores, small perithecia, and is closely related to No. 14. Nos. 17, 18 and 19 represent the types of *P. carpinicola* E. & E., *P. Juglandis* E. & E. and *P. Shepherdiae* Pk. respectively. They all have large perithecia and three-septate spores, and differ chiefly in slight differences in spore size. These spores, again, differ from such as those of *P. Thuemeniana* of the leptosphaeroid series chiefly in the greater diameter and more rounded ends. No. 79 is *Pyrenophora moravica* Petr., with irregularly seven- to 11-septate spores which are intermediate between Nos. 64 and 72.

The collections of the *vulgaris* series with asymmetrically septate spores are interesting in that they all seem derived from similar forms with symmetric septation by the addition of a septum in the lower end (or perhaps the dropping out of a septum in the upper half). They all show the rounded ends of this series. The type of *P. kansensis* E. & E. (No. 24) has four-septate yellow-brown spores (FIG. 35) which might be derived from such as are found in No. 14 by the insertion of a septum in the lower end cell. The type collection of *P. infectoria* var. *nigriseda* Rehm (No. 87) has similar four-septate spores, but they are dark red-brown, and many spores are three-septate. Nos. 25 and 26 represent misdetermined collections of *Pyrenophora phaeocomoides* and *Pleospora herbarum* (FIG. 36) respectively with rather dark brown to red-brown four-septate spores, much larger than in No. 87. No. 25 appears to be *P. richtophensis* in which no secondary septum was laid down in the upper half. In fact some perithecia show many spores which are five-septate and show such septation. Nos. 27, 28 and 29 are all six-septate by the addition of a cross-wall in each end of the spore. No. 28 is a collection labeled *P. oligotricha* Niessl, which has rather small yellow-brown six-septate spores, which appear as *vulgaris* spores with an extra septum in the lower half. No. 27 (FIG. 38) represents several collections with larger red-brown spores similarly related to *P. richtophensis*. No. 29 (FIG. 37) includes collections of *P. orbicularis* Auersw. (*P. Berberidis* Rab.) which

have six-septate spores with a characteristic clavate form, the upper portion being strongly rounded and the lower long-tapered.

HERBARUM SERIES

At the five-septate level of the *vulgaris* series certain changes occur in spore form and septation which give rise to the *herbarum* series. This area of transition from the *vulgaris* to the *herbarum* spore type is the most difficult one in the genus because the species here concerned are the most abundant and widely distributed and consequently show all sorts of character combinations and overlapping variations. The result is an evenly graded series of changes in which specific limits must be chosen in a purely arbitrary manner. The changes which occur to give the *herbarum* type of spores may be enumerated as follows:

1. The spore becomes even broader and with more rounded ends, resulting in an oblong-ellipsoid shape.
2. There is a tendency for the spores to be more asymmetric but less inequilateral or curved, becoming straight with a longer, narrower, tapered lower end.
3. Vertical septa are formed in the end cells. These often first appear as branched "Y" shaped septa, thereby also increasing the number of cross septa.

As the transition from *P. vulgaris* (No. 33) or its red-brown variety (No. 38) to the corresponding *P. herbarum* (No. 49) or its var. *occidentalis* (No. 43) is a gradual one with all sorts of variants, it is necessary to choose some arbitrary character to limit *P. vulgaris* and the *vulgaris* series. The presence of vertical septa within the end cell of at least a fair percentage (10-100%) of the spores has been taken as such a criterion.

This large group of collections, with spores five- to seven-septate, $14-28 \times 7-12 \mu$, and always with some spores with vertically septate end cells, contains the type collections of a dozen or more described species, but probably lies nearest to the *P. media* of Niessl, which he states (1) differs from *P. vulgaris* only in the darker color and vertically septate end cells. This group is divided into a yellow-brown spored variety (No. 34) and one with red-brown spores (No. 39) which are somewhat larger. In this group, some

collections will show small, yellow-brown, broadly rounded, five-septate spores with vertically septate end cells (as in FIG. 39a); others will have larger, red-brown, fusoid-ellipsoid, six- or seven-septate spores (39b); large, red-brown, broadly ovoid, seven-septate spores (39c) or many other combinations of these characters.

This series continues in an unbroken fashion, with all possible variants, through the many collections commonly referred to *P. herbarum* (Fr.) Rab. There have been a score or more of species described upon the basis of collections falling within the range of *P. herbarum* as here outlined. Although occasional collections seem to be distinct, no group of collections can be found which appears distinct enough to be called a species. *P. herbarum* is here arbitrarily limited to those species with spores having five to seven, mostly seven, septa, and which measure $22-40 \times 9-14 \mu$. It will be noted that the spore measurements of *P. media* (No. 34 and No. 39) overlap those of this group. However, collections of *P. media* with spores longer than 22μ will always show some shorter than this, whereas collections of *P. herbarum* with spores shorter than 26μ will always show some which are longer than this.

The collections of *P. herbarum* on leaves are represented on the chart as No. 48. Many of these collections have small perithecia ($100-200 \mu$), somewhat smaller spores and a high percentage of five-septate spores. On the other hand, such forms also occur upon stems, and some collections on leaves show large perithecia and larger, seven-septate spores. These may, therefore, represent merely a doubtful habitat grouping.

Two varieties, No. 49, with yellow-brown spores, and No. 43, with red-brown spores, are again recognized. Figure 40a is taken from the type of *P. Herniariae* Fck., on leaves. Figure 40b is from Rab. Herb. Myc., Ed. I, No. 547a, and represents the yellow-brown asymmetric spore type common in most European collections as well as in many others, and which are placed on the chart as No. 49. Figure 40c is characteristic of the red-brown type of spore commonly found in the mountain regions of western America and described by the writer as var. *occidentalis* (No. 43). This variety often shows more inequilateral spores.

In the larger spores of *P. herbarum* and throughout the spores of the rest of this series, the number of vertical septa laid down

increases, so that there are often from two to three or more vertical septa visible in face view between any two transverse septa. Such vertical septa usually extend continuously through several or even all cells, but this is not always true by any means.

The upper limit of spore size for *P. herbarum* is arbitrarily set at $40 \times 14 \mu$. There are, however, many collections with similar spores but of greater size ($30\text{--}55 \times 16\text{--}20 \mu$). Many collections show small spores which fall within the *P. herbarum* range, but also spores larger than $40 \times 14 \mu$. Such collections appear under a number of binomials and are grouped here under No. 66. The type collections of *P. maritima* Rehm (FIG. 42), *P. Jaapiana* Rehm (FIG. 41), *P. Armeriae* (Rab.) Ces. & deNot, and *P. Balsamorhizae* Tracy & Earle fall in this group. Some of these collections show rather characteristic spore forms but further collections are needed to determine whether these are constant.

The next change in septation, after the seven-septate *P. herbarum* type, is the appearance of irregular septa in the lower end of the spore at angles other than right angles to the spore axis. This results in an irregular fourth septum in the lower end, giving an eight-septate spore (FIG. 43). Such spores were previously referred to *P. stenospora* (5), but the type collection of *P. coloradensis* E. & E. also has them. These collections are represented on the chart as No. 60. These spores are red-brown and asymmetric, the lower half always being longer, narrower and somewhat tapered. This asymmetry of both form and septation is characteristic of all the remaining species in this series.

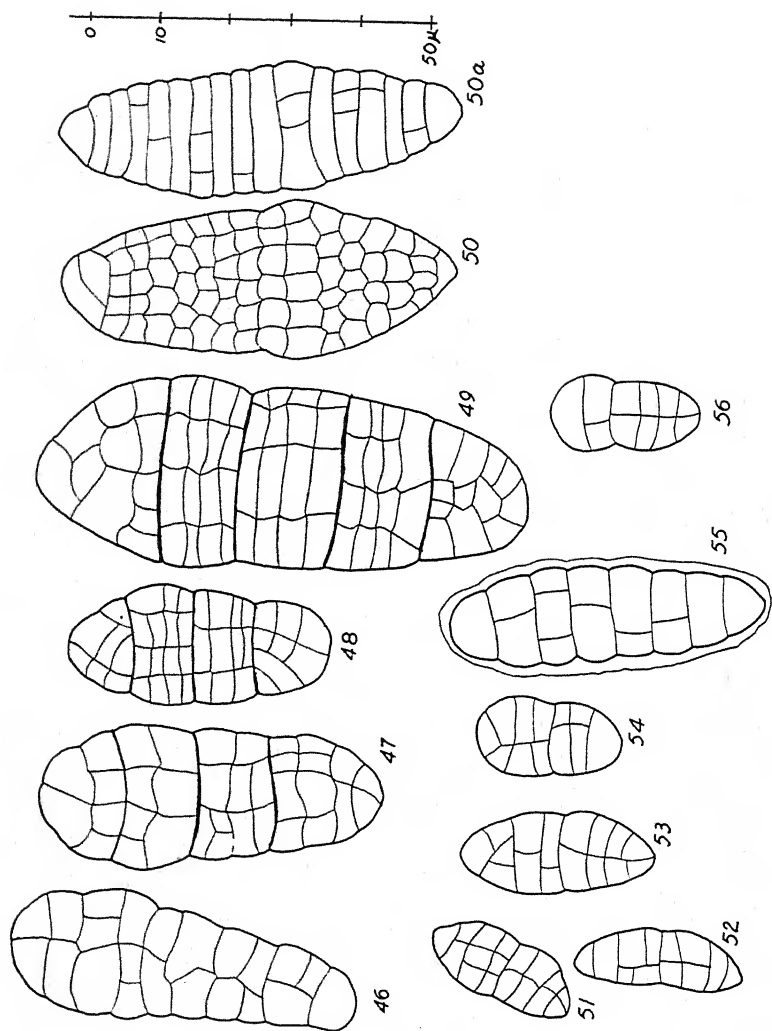
It is necessary here to examine the sequence of formation of the transverse septa. If the first three septa laid down be considered as primary, the five-septate spore (of this series) arises by the formation of secondary septa in the two central cells and the seven-septate spore by the additional septa in the two end cells. In No. 60, an additional eighth septum is irregularly laid down in the lower end giving rise to an asymmetric septation. The next step, found in No. 61 (FIG. 44), is the insertion of one or two regular cross walls in the lower end of the spore, giving one with eight or nine septa. Sometimes the formation of irregular oblique septa (as in No. 60) in either end makes these spores appear ten- to eleven-septate. The writer has collections of this type only from the

Rocky Mountain region. The numerous collections reported by Petrak (2, 3) as *P. chlamydospora* Sacc. on *Astragalus* from the mountainous regions of Iran also seem to belong to this group.

The next change in septation is a distinct one and is seen in *P. montana* Wehm. (No. 62) (FIG. 45). The spores show three septa above and usually six to seven septa below the primary septum. This septation is the result of the formation of tertiary septa in all of the cells in the lower half of a seven-septate spore. In *P. montana* this origin is not so obvious because the septa are regularly laid down, but in another series of collections, shown as a lateral branch to the left of those on herbaceous stems in the *herbarum* series on the chart, the septa are tardily and incompletely formed and this manner of origin is more strikingly shown.

The origin of this irregular septation is found in No. 67, which includes the type collections of *P. laxa* Ell. & Galw. and *P. longispora* Speg. (FIG. 46). The spores of these species are yellow-brown and basically seven-septate, but incomplete, tertiary, cross-septa are commonly found laid down between two neighboring vertical septa but do not extend across the spore. Such septa are usually in the lower half of the spore, but give rise to irregularly eight- to nine-septate spores. The type of *P. pulchra* Kirschst. (No. 73) shows a continuation of this tendency, the irregular cross septa here being more numerous and formed in the upper as well as the lower half of the spore, resulting in ten to fifteen transverse septa.

No. 57 represents a group of collections with red-brown spores showing extremely variable septation. The first three primary septa in these spores are usually thicker and more prominent. The secondary and tertiary cross walls laid down in the two central cells are thinner, often tardily formed, and irregular or incomplete, and extend between only two of several vertical septa. The two primary end cells in these spores tend to be irregularly septate by oblique septa. As a result, the septation in spores of a single mount may vary from seven to fifteen cross septa. Figure 47 is a spore of this type which might be referred to *P. montana* if it were not for the irregular septation. Figure 48 is a spore with a more complete septation, showing the three transverse septa in each of the central primary cells and the oblique septation of the end cells.

FIGS. 46-56. Spores of *Pleospora*.

The type collection of *P. alpestris* E. & E. is of this type, as is also a collection labeled *P. multiseptata*, on *Anthyllis*, which suggests that *P. Anthyllidis* Niessl is of this type.

The type collection of *P. amplispora* E. & E. (No. 65) has very large red-brown spores (FIG. 49) showing this type of three secondary and tertiary cross septa in one cell above the primary central septum and in two cells below this septum. The end cells are again obliquely septate in an irregular fashion. This results in spores with thirteen to eighteen or even more septa.

The type collection of *P. multiseptata* Starb. (No. 74) has very pale yellow-brown spores with fifteen or more transverse septa and are broken up into small cubical cells by numerous vertical walls.

No. 91 represents an undetermined collection on *Stipa*, which has somewhat flattened spores and which may belong in *Clathrospora*. In face view (FIG. 50) they have fifteen to sixteen transverse and five to six vertical walls which are very regularly formed. In edge view (FIG. 50a) the spores are narrower and show only scattered vertical walls which appear comparatively faint.

No. 59, which is shown as an offshoot of the herbaceous *herbarum* line, includes several western collections already referred to *P. obligasca* Bub. The spores (FIG. 56) are asymmetrically four- to six-septate, and although similar to this series in form, they are of a more simple type and do not seem to fit in with the rest of the series.

The *P. herbarum* types on woody stems are mostly at the seven-septate level and correspond to the *P. media* (Nos. 34 and 39) group. No. 51 refers to a group of collections with small, often clustered perithecia on shrubby hosts, and includes the type collections of *P. rubicola* Syd., *P. Ephedrae* Speg. and *P. Rhodotypi* Rehm (inedit.). The spores (FIG. 51) are broadly fusoid-ellipsoid and irregularly septate.

No. 47 refers to a group of collections on decorticated woody stems which were previously placed in the genus *Teichospora*. They were transferred to the genus *Pleospora* by Höhnelt (4, p. 230) because the perithecia actually originate beneath the surface of the wood. The spores (FIG. 52) are almost identical with those of No. 51, although they tend to be somewhat narrower in comparison to their length. This group shows the close relationship between the

woody forms of *Pleospora* and the genus *Teichospora*. The separation of the two genera will probably need revision after a study of these related forms.

No. 89 represents a group with very similar spores (FIG. 53) but much larger perithecia and found within the bark of woody stems. It includes the type collections of *P. laricina* Rehm and *P. pustulans* E. & E.

No. 11 is a collection of *P. Thuemeniana* Sacc., on *Agave*, which has a blackened clypeus about the large perithecia and has spores (FIG. 54) similar to the preceding, but shorter, stouter and with more rounded ends.

No. 80 is *P. Henningsiana* Ruhl, Jahn & Paul, which is a distinct species, with large perithecia and seven-septate, elongate, cylindric spores with a gelatinous envelope (FIG. 55).

There are numerous collections with setose perithecia which have spores corresponding to the *herbarum* series. On herbaceous stems the perithecia are usually larger and more or less covered with a dense tomentum of stiff sinuous hairs. On the upper surface of such perithecia the hairs tend to become stiff, upright, pointed and to penetrate the overlying tissues. If soon freed from these overlying tissues these stiff hairs develop more abundantly, but they are easily broken off and it is a common experience to find only a few perithecia in a collection which show such setae. On leaves or minute stems there is another group in which the young perithecia are elongate-pyriform and have a rather prominent stout ostiolar neck from which there arise a cluster of pointed setae, whereas the base remains smooth. Such setose perithecia are usually found at high altitudes and are to be found under a multitude of binomials in herbaria. In Europe they are commonly referred to *P. helvetica* Niessl or *P. hispida* Niessl.

The spores of such collections show the same continuous series of overlapping variation which has been described for the *P. media-P. herbarum* series. By using a rather loose correlation of spore and perithecial characters an attempt has been made to separate certain specific (or varietal ?) groupings. Nos. 90, 53, 54 and 84 are a closely related group with five- or seven-septate spores with septate end cells, $19-26.5 \times 7-10.5 \mu$, varying from yellow- to red-brown and from fusoid- to oblong-ellipsoid. In No. 90 the spores

are of the *vulgaris* type, similar to No. 81 but smaller and with vertically septate end cells. Nos. 53 and 54 both include collections with yellow-brown spores, which are more generally tapered in the former and with rounded ends in the latter. No. 84 includes forms with red-brown spores of variable form.

No. 56 represents a group of collections which show a similar spore-form but with spores slightly longer ($21-28 \times 9-12 \mu$), more oblong, and nearly always with seven septa. They are yellow- to red-brown.

No. 50 includes those collections with larger spores ($26-37 \times 11-16 \mu$) which correspond to *P. herbarum*. The spores of these collections are mostly dark yellow- to dark red-brown and often have the inequilateral form of the var. *occidentalis*, which is probably correlated with their occurrence at high altitudes.

No. 58 is the culmination of this series. In these collections the spores run still larger and show the irregular septation in the lower end with insertion of an eighth septum, as is found in No. 60.

No. 55 represents the type collection of *P. abbreviata* Fck., which shows spores with the irregular type of tertiary cross wall formation mentioned for No. 57. The perithecia are, however, of the conic, setose, leaf inhabiting type.

Many of the collections with setose perithecia are found on leaves. Most of the leaf inhabiting individuals, with spores of the *herbarum* series, are not distinct enough to be considered as separate groupings. No. 44, which is the type of *P. Inulae-candidae* Jaap, has the spores of the *P. media* (No. 39) type, but with the small perithecia imbedded in the dense mat of leaf hairs on the leaves of *Ipula candida*. No. 45 consists of two collections, in Riksmuseet, labeled *P. phyllophyla* Rehm (inedit.) and has similar spores which, however, show very little or no constriction at the septa.

It is intended that the rather preliminary outline here presented will be supplemented in future papers by a more detailed presentation of the individual collections and species groups which will make possible the delimitation of species and application of binomials.

It can be seen, however, that there are certain tendencies which persist and are correlated with other varying factors which allow

the detection of related groups. The primitive type of septation which gave rise to the genus, for instance, was the occasional insertion of a vertical septum. This is found only in yellow-brown spores of rather narrow fusoid or oblong form. It persists during the formation of secondary septa and a five-septate spore, but as more septa are laid down (*i.e.*, in the asymmetrical leptosphaeroid branch) the *vulgaris* type of secondary septation is likely to occur.

This *vulgaris* type of secondary septation may occur in three-septate spores and is usually accompanied by a broader, inequilateral or curved spore form, which persists, without vertical septation of the end cells, even though nine to eleven transverse septa are formed.

Again, the *herbarum* series arises at the five-septate level by the further broadening of the spore and rounding of the ends. This rounding of the ends results in an increase in diameter of the end cells, which probably allows the formation of vertical septa in these cells, which in turn is characteristic of this series. As this type of spore increases in size, secondary and tertiary cross walls are formed until fifteen to eighteen transverse septa are found. The asymmetry of form of these spores with a longer lower end apparently arose first (in seven-septate spores) and was followed by the formation of additional septa in the lower portion, giving the asymmetrical septation characteristic of most of the members with numerous cross walls. This asymmetry as regards the formation of cross walls also occurs in both of the other series, but seems to be merely occasional rather than the dominant type of septation as it is in the larger spores of the *herbarum* series.

The clathrate spore forms (*Clathrospora*) are not discussed here, but it might be mentioned that they also show several distinct types of spore form, with a similar overlapping series of collections as regards spore size and septation for each.

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EXPLANATION OF FIGURES

FIG. 1. Basic spore form of the genus.

FIGS. 2-5. Fundamental modifications in spore form: 2, showing constrictions at septa; 3, inequilateral modification; 4, showing rounding of ends; 5, asymmetric development.

FIGS. 6-11. Generalized spore forms of the genus: 6, fusoid (leptosphaeroid type); 7, fusoid-ellipsoid (*vulgaris* type); 8, oblong-ellipsoid (*herbarum* type); 9, clavate, asymmetric type; 10, oblong type; 11, 11a, flattened clathrate (*Clathrospora* type).

*FIGS. 12-14. Leptosphaeroid (fusoid, symmetric) types: 12, spore of *Pleospora pellita* (Fr.) Rab.; 13, spore of undetermined collection (*P. vagans* Niessl), on *Phleum*; 14, spore from type collection of *Leptosphaeria Yerbæ* Speg.

FIGS. 15-17. Leptosphaeroid (oblong) types: 15, spore from type collections of *P. mollis* Starb.; 16, spore from collection of *P. oblongata* Niessl, in the Rehm Herb.; 17, spore from another collection of *P. oblongata*, in Rehm Herb., labeled as a variety.

FIGS. 18-21. Leptosphaeroid (fusoid, asymmetric) types: 18, spore from type material of *P. Niessleana* Kze.; 18a, spore from collection of *P. dura* Niessl, on *Linaria*; 19, spore from collection of *P. dura*, on *Aconitum*; 20, spore from collection of *P. abscondita* Sacc. & Roum., on *Phragmites*; 21, spore of an undetermined collection on *Sorbus*.

FIGS. 22-23. Leptosphaeroid (fusoid-ellipsoid) types: 22, spore of an unpublished species, *P. Galii* Romell; 23, spore of an undetermined collection, on *Galium*.

FIGS. 24-34. Spores of the *vulgaris* series (symmetric types): 24, spore from type material of *P. oligostachyæ* E. & E.; 25, spore of a collection of *P. diaporthoides*, on *Bardana*; 26, spore from type collection of *P. Boldoæ* Speg.; 27, spore from type material of *P. infectoria* Fck.; 28, spore from type collection of *P. infectoria* var. *Sacchari* Speg.; 29, spore from a collection of *P. richtophensis* E. & E., on *Achillea*; 30, spore from a collection of *P. rubicunda* Niessl, on grass; 31, spore from collection of *P. Thurgoviana* Wegelin, on *Typha*; 32, spore from type collection of *P. Alismatis* E. & E.; 33, spore from collection of *P. Anthyllidis* var. *Aconiti* Rehm; 34, spore from type collection of *P. pleosphaeroides* Wehm.

FIGS. 35-38. Spores of the *vulgaris* series (asymmetric types): 35, spore from type collection of *P. kansensis* E. & E.; 36, spore from a collection of *P. herbarum* (Clem. Cr. Form, Colo. No. 442); 37, spore from a collection

* Figures 12-56 were drawn with a camera lucida, to the scale indicated on the plates.

of *P. Berberidis* Rab., in the Rehm Herb.; 38, spore from an undetermined collection on *Arenaria*.

FIGS. 39-45. Spores of the *herbarum* series (on herbaceous stems): 39a, spore from a collection of *P. oblongata* Niessl; 39b, spore from a collection of *P. Maireana* Lamb. & Fautr., on *Laserpitium*; 39c, spore from a collection of *P. Compositarum* Earle, on *Penstemon*; 40a, spore from type collection of *P. Herniariae* Fck.; 40b, spore of *P. herbarum* (Fr.) Rab., from Rab., Herb. Myc., Ed. I, No. 547a; 40c, spore taken from the type of *P. herbarum* var. *occidentalis* Wehm.; 41, spore from type collection of *P. Jaapiana* Rehm; 42, spore from type collection of *P. maritima* Rehm; 43, spore from collection of *P. stenospora* Schroet., on *Rydbergia*; 44, spore from a collection of *P. nejagusensis* Bub., on *Hedysarum*; 45, spore from type collection of *P. montana* Wehm.

FIGS. 46-49. Spores of the *herbarum* series (on herbaceous stems, with irregular septation): 46, spore from type collection of *P. longispora* Speg.; 47, spore from a collection of *P. montana* Wehm., on *Lupinus*; 48, spore from a collection of *P. multiseptata* Starb., on *Anthyllis*; 49, spore from the type collection of *P. amplispora* E. & E.

FIGS. 50, 50a. Face and edge views of a spore from an undetermined collection, on *Stipa*, showing flattened character of spores of the genus *Clathrospora*, but with vertical septa in edge view.

FIGS. 51-55. Spores of the *herbarum* series (on woody stems): 51, spore from type collection of *P. rubicola* Syd.; 52, spore from a collection of *Pleospora obtusa* (Fck.) Höhn., f. *fibrincola* Höhn., on pasteboard; 53, spore from the type collection of *P. laricina* Rehm; 54, spore from a collection of *P. Thuemeniana* Sacc., on *Yucca* or *Agave*; 55, spore from a collection of *P. Henningsiana* Ruhl, Jahn & Paul, on *Salix*.

FIG. 56. Spore from Clem. Crypt. Form, Colo. No. 34 (*P. cybospora* Clem.), showing a simple type of asymmetric septation, with the *herbarum* form.

SOME LEAFSPOT FUNGI ON WESTERN GRAMINEAE—III

RODERICK SPRAGUE¹

(WITH 2 FIGURES)

The fungi listed or discussed in this article were obtained in the far western United States during 1947. About eight hundred specimens were collected by G. W. Fischer, Jack Meiners, and the writer in Idaho, Utah, Arizona, New Mexico, California, Nevada, Oregon, and Washington during June 1947. In addition, the writer collected about 90 numbers in the Chelan National Forest north of Winthrop, Washington. Although the areas covered were extensive, the weather conditions, except in the Chelan region, were not favorable for leafspot development this year because of the shortage of seasonal rainfall. The following fungi are worthy of note:

Ascochyta utahensis sp. nov. Maculis brunneis, centro pallido; pycnidiiis globosis, brunneis, pseudoparenchymatis, epiphyllis, ostiolatis, 113-148 μ ; pycnosporulis elongato-ellipticis, cylindraceo-irregularibus, apicibus et basibus subobtusis, 1-septatis, contextu hyalino-chlorino, guttulado v. granuloso, 22.6-29 \times 6.6-10 μ .

Hab. in foliis vivis et dejectis *Agropyri inermis* (Scribn. et Smith) Rydb., prope Logan, Utah. June 6, 1947. Sprague, Fischer, et Meiners coll. **Typus** est C. S. 3610² (Wash. State Coll. Dept. of Plant Pathology herbarium).

Spots chocolate brown, 1-6 \times 1-3 mm., mostly elongate or forming irregular areas by fusion of several spots; pycnidia few, scattered, obscure, pseudoparenchymatous, brown, globose, ostiolate, 113-148 μ ; pycnosporules elongate-elliptical or cylindrical but tapering to blunt ends, straight or more often bent or slightly lumpy in outline, contents yellow to nearly hyaline, guttulate to granulose, one-septate (median septation), not or scarcely constricted at the septum, large, 22.6-29.2 \times 6.6-10 μ .

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² C. S. refers to the Collection Series of the pathological collections of the Department of Plant Pathology, Washington State College, Pullman.

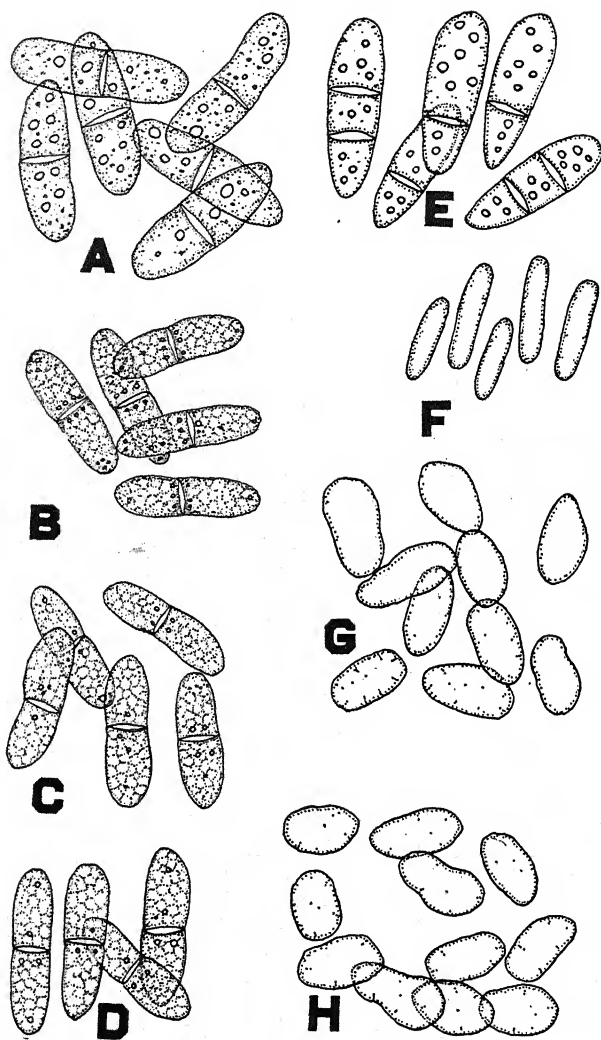


FIG. 1. Pycnospores of fungi on Gramineae.

This material was obtained on a steep, grassy slope in Logan Canyon near Logan, Utah, and consists of a number of leaves with well-defined spots which could be mistaken for those caused by *Pseudomonas coronafaciens* var. *atropurpureum* (R. and G.) Stapp. The pycnidia are scarcely discernible with a hand lens and are not

abundant. The spots which are brown when young eventually become pallid in the center.

This species has larger spores (FIG. 1, *a*) than any of the other species of *Ascochyta* on western grasses. In the writer's key to the species³ it belongs in section *Ascochyttula* and is probably closest morphologically to *Ascochyttella avenae* Petrak.⁴ However, even in winter material, the spores of the latter species measure only $17-26 \times 6.2-7.0 \mu$, and, therefore, just exceed the minimum dimensions of those of *A. utahensis*. In addition, the symptoms of the latter appear very distinct from those of *A. avenae*.

A. utahensis was compared with *A. agropyrina* (Fairman) Trotter (9). This species, which occurs on *Elymus* and *Agropyron* in the Great Plains, has yellow spores measuring $14-19 \times 4.5-6.2 \mu$ in summer material. The spores of the latter are, therefore, much smaller and also tend to be more uniformly cylindrical. It is doubted if *A. utahensis* could be a giant phase of *A. agropyrina*. Therefore, it is described as distinct.

This fungus, collected during the Centennial commemorative year of the colonizing of Utah, is named for that state. One collection on *Agropyron smithii* Rydb. made near Belfield, North Dakota, June 19, 1945, is apparently the same species (*B. P. I.* 81, 124).

ASCOCHYTA HORDEI Hara. Hara has described (12) a species of *Ascochyta* on barley from Japan which is the same as a yellow-spored species from the western United States (FIGS. 1, *b*; 1, *c*; and 1, *d*).

Material on *Hordeum murinum* L. from near Riggins, Idaho (*C. S.* 3677), on *Bromus carinatus* Hook. and Arn. from Logan,

³ Sprague, R. Rootrots and leafspots of grains and grasses in the northern Great Plains and western states. U.S.D.A. B.P.I.S.A.E. Plant Disease Reporter. Supplement 163. June 15, 1946. (Processed.)

⁴ Ann. Myc. 23: 107. 1925. This will probably be discussed in a later publication. *Ascochyttella avenae* should be known as ***Ascochyta avenae*** (Petrak) Sprague and A. G. Johnson comb. nov. Spots usually vague necrotic areas, pycnidia prominent, dark brown, $100-140 \mu$ (in western United States); pycnosporos irregularly cylindrical to fusoid; ends rounded, spore contents coarsely granular, sometimes two-septate but usually with one median septation, $17-26 \times 6.2-7 \mu$, tardily yellow. Spores sometimes smaller in summer material.

Utah (C. S. 3852), and on *Festuca elatior* L. near Logan, Utah (C. S. 3656 and 3682) carried what we call *Ascochyta hordei* with the following characters:

Spots few, whitish yellow to clay colored or vaguely pale, nearly white, later with brown margins or sometimes fading to vague necrotic areas; pycnidia thin-walled, parenchymatous, globose or slightly flattened, ostiolate, epiphyllous, erumpent, $100\text{--}172\ \mu$; pycnosporos chlorinous or very pale yellow to virtually hyaline, cylindrical with rounded ends or less often slightly twisted or semi-fusoid but scarcely fusoid and not tapering strongly towards either end, one- (seldom two)-septate, $16\text{--}22 \times 4.8\text{--}6.2\ \mu$ on *Festuca* (FIG. 1, b) and *Hordeum* (FIG. 1, c); $17.8\text{--}21 \times 5.3\text{--}7.1\ \mu$ on *Bromus* (FIG. 1, d).

APIOCARPELLA MACROSPORA (Speg.) Sydow (*Apiospora macrospora* Speg.). Somewhat scanty material of this probably saprophytic species was collected on *Stiporyzopsis bloomeri* (Bohland) Johnson [*Oryzopsis hymenoides* (Roem. and Schult.) Ricker \times *Stipa occidentalis* Thurb.] at 8,000 feet on Mt. Whitney, California (C. S. 3605). The prominent, black, brittle pycnidia were in straw-colored areas on dead leaves. The pycnidia were at first immersed, but later, through erosion of the leaf epidermis, were superficial on the sub-epidermal layers. They were very flattened, about $80\ \mu$ tall, $160\text{--}350\ \mu$ long and $80\text{--}130\ \mu$ wide and had no ostiole until nearly mature. The spores were elliptical to elongate (narrowly obovate), with the basal (smaller cell) definitely pointed (FIG. 1, e). The eccentrically placed cross-wall is typical of the species. The spores were slightly greenish-hyaline with a number of medium-sized bodies in the cell contents, $21\text{--}26 \times 6.6\text{--}8.8\ \mu$. Spegazzini (17) described this species as *Apiospora macrospora* in 1910, but, as the genus name had been used by v. Höhnelt in 1909, Sydow (23) proposed *Apiocarpella*. Spegazzini described the species on *Hordeum jubatum* as having lenticular, prominent pycnidia, $150\ \mu$ in diameter, with coarse parenchymatous tissue, and ellipsoid-elongate conidia $28\text{--}30 \times 7\text{--}8\ \mu$, and with the lower cell of the spore much smaller than the upper. Without having the type available for critical comparison it seems conservative to consider our fungus the same as Spegazzini's, as the spores, except for being slightly shorter, have approximately the same dimensions.

H. C. Greene sent a specimen of *Apiocarpella* on *Calamagrostis canadensis* L. from Madison, Wisconsin, collected July 22, 1947. The fungus occurred in elongated, pale spots on living leaves. The spores were $13-16 \times 3.7-5.0 \mu$ and therefore were much smaller than those of *A. macrospora*. This fungus will be described as an autonomous species in one of Greene's papers on Wisconsin fungi.

***Pyrenochaeta elymi* sp. nov.** Pycnidii subgregariis, erumpentibus superficialibus, globosis, ostiolatis, aureo-brunneis, v. brunneis prope ostioli, contextu membranaceo-parenchymatico, $130-170 \mu$ diam.; setulis 5-15, rigidis, septatis, acutis, socia ostioli, $120-170 \mu$; pycnosporulis numerosis, continuis, hyalinis, cylindraceis, $13.2-15 \times 2.6-3.4 \mu$.

Hab. in foliis dejectis, *Elymi glauci*, prope Logan, Utah.

Pycnidia several in gray, dead tissue, subgregarious, subsuperficial, globose, ostiolate, golden brown but with a contrasting dark brown ring around the slightly rostrate ostiole, membranaceous-parenchymatous walled, composed of squared cells as viewed in whole mount, $130-170 \mu$ diameter but young pycnidia sometimes smaller; setae numerous, 5-15 in number, ringing ostiole and rising above pycnidium to a distance approximately equal to the height of the pycnidium, somewhat divergent, brown, septate, tapering to a point, rigid and spine-like, up to 170μ long; pycnospores numerous, exuding in tendrils from the ostioles, hyaline or slightly smoky in mass, cylindrical but with rounded ends (bacillar) all nearly identical in size and shape, $13.2-15 \times 2.6-3.4 \mu$ (FIG. 1, f).

Hab. on dead leaf tips of *Elymus glaucus* in Logan Canyon, near Logan, Utah (C. S. 3631). Collected by Jack Meiners, G. W. Fischer and R. Sprague, June 6, 1947.

This interesting saprophyte appears to be distinct from any known species of this genus on grasses or sedges. *P. exosporioides* (Fckl.) Sacc. (15, v. 3, p. 221) has elliptical spores $10 \times 3 \mu$. It occurs on *Carex*. The shape of the spores, if not their size, removes *P. sp.* from this species. *P. luzulae* (West.) Sacc. (*Vermicularia luzulae* West.) has globose spores 5μ in diameter, and is even more distinct. *P. leptospora* Sacc. and Briard (16) has oblong to ovoid, cylindrical or obtuse spores, $4-5 \times 1.5 \mu$, and occurs on *Milium effusum* L. *P. graminis* Ell. and Ev. (8) has pycnidia without ostioles and has short setae $20-40 \times 3 \mu$, as well as globose or ovate spores $8-14 \mu$ long. It occurs on dead foliage of *Chloris verticillata* Nutt. *P. oryzae* Miyake (14) is

closer to our material than most of the others in pycnidial characters, but the spores are fusoid, $4-6 \times 1.5-2 \mu$.

Macrophoma sporoboli sp. nov. Pycnidiiis non in maculis v. in maculis griseis, epiphyllis, erumpentibus, nigris, depresso-globosis v. ellipsoideis, carbonaceo-parenchymatis, ostiolatis (minute et tarde), $70-155 \times 70-140 \mu$; pycnosporulis hyalinis v. opacis, aseptatis, variabilibus, ellipsoideis, v. subcylindraceis, obovatis, subfusoides, $7.5-19 \times 5.6-8.9 \mu$ ($13-17 \times 6.6-7.8 \mu$).

Hab. in foliis emortuis *Sporoboli gigantei* Nash, prope Winslow, Arizona. Sprague, Fischer et Meiners, coll. June 11, 1947. **Typus** est C. S. 3609; et in *Sporobolo* sp. Hatch, New Mexico. Sprague, Fischer et Meiners, June 13, 1947 (C. S. 3670).

Pycnidia not in spots, or sometimes in gray lesions, epiphyllous, scattered, black, depressed-globose to less often somewhat ellipsoid, erumpent, carbonaceous-parenchymatous, $70-155 \times 70-140 \mu$, ostiole minute or often very tardily formed; spores released in mounts by crushing the brittle pycnidia, pycnosporules hyaline to somewhat opaque but not tinted (when examined six weeks after collecting), non-septate, variable in shape, generally elongate-ellipsoid or tapering towards one end and then obovate, sometimes ovate to nearly truncate, sometimes nearly cylindrical, a few small spores subglobose, others sub-fusoid, or even constricted towards one end and then roughly broadly pyriform, $7.5-19 \times 5.6-8.9 \mu$ but typically $13-17 \times 6.6-7.8 \mu$.

On dead leaves of *Sporobolus giganteus* Nash, 5 miles east of Winslow, Arizona (C. S. 3609, **Type**), and on *Sporobolus* sp. Hatch, New Mexico (C. S. 3670).

The type appears to be one of several saprophytes on the leaves of the conspicuous giant drop-seed growing along the main highway east of Winslow. A species of *Leptosphaeria* is associated with the fungus.

At the time examined, this fungus had hyaline spores (FIGS. 1, g; 1, h) with the typically opaque, non-guttulate contents of the spores of fungi which we have placed in *Macrophoma*. There was no indication of color (*Sphaeropsis*) in the spores at the time examined. However, it might be open to question as to why this should not be placed in *Phyllosticta*. The spores, we believe, are somewhat large for *Phyllosticta*, and, in addition, the absence or vagueness of spots is possibly more like *Macrophoma*. Furthermore, the opaque (or "frosty"), non-guttulate aspect of the spore contents is nearer

to that of *M. phlei* Tehon and Stout, for instance, than to that of *P. rogleri* Sprague, a large-spored *Phyllosticta*. *M. sporoboli* differs from *M. phlei* in having distinctly smaller polymorphic spores. Although many of the spores are typically elongate to egg-shaped, others vary from pyriform to nearly globose.

The material on *Sporobolus* sp. from Hatch, New Mexico, had gray, vague, ellipsoidal spots, $2-4 \times 0.5-1.5$ mm. The pycnidia were black, subimmersed in lines, globose, non-ostiolate (? immature, scarcely *Sclerophoma*), $120-145 \mu$ in diameter, and the spores had the same multiplicity of shapes as found in the type of *M. sporoboli*. In the Hatch material they measured $11-16 \times 6.6-8.9 \mu$ (FIG. 1, *g*). Therefore, the two collections are apparently co-specific, the only real difference being in the absence of a definite ostiole in the Hatch material. Ostioles are also sometimes absent or vague in the Winslow material, appearing to be scarcely more than a localized decadence of the pycnidial dome (compare FIGS. 1, *g*, and 1, *h*).

SEPTORIA AVENAE Frank on FESTUCA ELATIOR L. *Festuca elatior* is seldom attacked by leaf-spotting fungi. Therefore, the presence of a dark purple spot on leaves of *Festuca elatior* L. from Logan Canyon, Utah, is of interest. The causal organism appears to be an *Ascochyta* stage of *S. avenae*. The spores are mostly one-septate, rarely two- to three-septate, cylindrical, hyaline, $16.8-27.2 \times 3.1-4.7 \mu$ (FIG. 2, *a*). They are too large for any phase of *Ascochyta graminicola* Sacc., and are perhaps not large enough for *Stagonospora arenaria* Sacc. (18). Such specimens as this one are too similar to summer material of *Septoria nodorum* Berk., *S. avenae*, *S. secalis* var. *stipae* Sprague and others to justify describing them as new. All of these, however, except *Stagonospora arenaria* and *Septoria avenae*, must be eliminated from consideration because of the width of the spores of the fescue material.

This material on *F. elatior* was found on a lush prairie slope above Logan Creek in Logan Canyon, Utah, where the host was associated with *Agropyron inerme* (cfr. *Ascochyta utahensis*), *Melica* sp., *Festuca kingii* (S. Wats.) Cassidy (cfr. *Oxularia hordei*), *Balsamorhiza* sp., *Symphoricarpos oreophilus* A. Gray, *Castilleja* spp., and other mountain plants.

Material of *S. avenae* Frank on *Glyceria pauciflora* Presl. was found in a boggy, wooded area on Andrew's Creek, Chelan National Forest, Washington, August 9, 1947 (C. S. 3871). The spots were gray, elliptical to striate, later fading to straw-color, and with wide surrounding yellow areas. A second collection from the same area (C. S. 3879) showed lesions which were similar, except that mature ones were nearly buff in color. The pycnidia were yellow-brown under a 16 mm. lens, very thin-walled, flattened-globose, up to 150μ in diameter, and ostiolate. Some of them were almost acervulus-like. This refers one to *Cylindrosporium glyceriae* Ell. and Ev. which has spores $15-30 \times 2.5-3\mu$. The status of this little known species is uncertain, however. Our material has true pycnidia, and the spores are cylindrical, three-septate, and $35-46 \times 3.4-4.1\mu$. The fungus appears to be close to, if not identical with, *S. avenae* Frank. The shaded, moist environment may have contributed to the fragile type of pycnidia. The fungus is distinct from our *S. glycericola* (22), which has smaller spores ($20-34 \times 1.9-3.4\mu$), with a distinctive, sub-acute apex. Spores of *S. avenae* on this host are truly cylindrical. *S. glycericola* produces suborbicular buff spots.

There is a char spot (? *Euryachora* sp.) with immature, black, globose fruiting bodies associated with *S. avenae* on this *Glyceria* material (C. S. 3872).

A collection on *Hordeum nodosum* L., made five miles south of Donnelly, Idaho (C. S. 3629), has bacillar one-septate spores, $15-23.6 \times 2.4-3.0\mu$, borne in brown, elliptical spots on living leaves. This appears to be *S. nodorum* Berk.

SEPTORIA ELYMI Ell. and Ev. Additional material of a *Septoria* on *Agropyron smithii* Rydb., from the vicinity of Logan, Utah (C. S. 3648), is assigned to the large, curved to sinuous form of *S. elymi* (FIG. 2, b). *S. elymi* proper has comparatively straight, stiff spores. However, when spores of this type elongate in a pycnidium they become curved or sinuous and often lose most of their resemblance to the smaller, straighter type. This appears to be the case with *S. elymi* as it occurs on *A. smithii* (North Dakota, Utah), *E. canadensis* var. *robustus* (Scribn. and Sm.) Macken. and Bush (Iowa), and *E. glaucus* Buckl. (Michigan). Davis (5) ten-

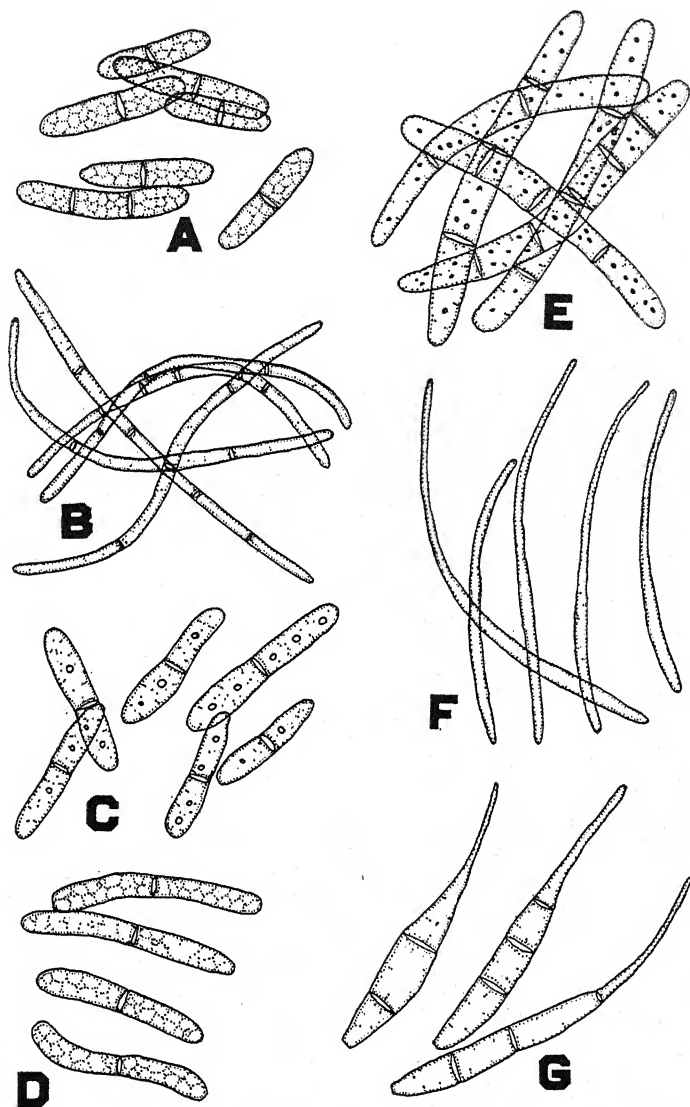


FIG. 2. Spores of fungi on Gramineae.

tatively described this form as *S. bromi* var. *clymina* on *E. virginicus* L. from Wisconsin. He retained this name in his final publication (7). Since this fungus tends to retain its large spore size in pure culture, it is possibly worthy of recognition. The writer

questions if this phase necessitates a formal name. But for those who prefer names for forms of species of fungi he proposes *Septoria elymi* forma *elymina* (J. J. Davis) comb. nov.

This phase of *S. elymi* differs from *S. pacifica* Sprague in having curved, not straight or stiffly curved, spores, in the type of growth on nutrient agar, and in the paler, very distinct pycnidia. It differs from *S. infuscans* (Ell. and Ev.) Sprague for the same general reasons. The complex on *Elymus* and *Agropyron* is difficult even for those critically studying the group.

S. agropyrina Lobik, *S. nodorum*, *S. avenae*, and *Stagonospora arnaria* all produce white cottony mycelium in pure culture, and their spores are coarser, ranging from coarsely obclavate in *S. agropyrina* to bacillar in *S. nodorum*. All occur, or have been reported, on *Elymus* and *Agropyron*. Frandsen (10) has recently described *S. elymina* (for *S. elymi* Rostr.), which has short, club-shaped spores and is very distinct from *S. elymi*.

SEPTORIA NODORUM Berk. on *FESTUCA SUBULATA* Trin. This is a locally common brown blight or leaf scald which covers a high percentage of the foliage of this grass growing in deep fir woods on Andrew's Creek in the Chelan National Forest north of Winthrop, Washington (C. S. 3789, 3880). Pycnidia are golden brown, globose, erumpent, 90–130 μ in diameter, and with small ostioles. The spores are hyaline, sub-cylindrical to fusiform-cylindrical, and straight, with somewhat blunted bases and tapered blunted apices. They are one-septate, slightly to scarcely constricted, and the contents are somewhat guttulate. They are 21–30 \times 3.1–4.8 μ , and hyaline but with faint tinting of the contents. The writer assigns this to *S. nodorum* with considerable hesitation, not because the spores are only one-septate, but because they are proportionately too broad and fusiform for typical spores of this species. The difference is apparent if spores of *S. nodorum* from *Festuca subulata* (FIG. 2, c) are compared with more typical ones of *S. nodorum* from *Elymus glaucus* Buckl. (also a new host; C. S. 3780, FIG. 2, d). However, *S. nodorum* is somewhat variable, especially on *Triticum aestivum* L., so it appears conservative to assign the material on fescue to that species. The spores of

S. nodorum on *F. subulata* are the same size as those of *S. avenae* on *F. elatior* (FIG. 2, a) but less cylindrical.

In addition, W. B. Cooke collected necrotic material of *Melica californica* Scribn. which had scattered pycnidia of *S. nodorum* on the leaves. The spores were *Ascochyta*-like, sub-cylindrical, one-septate, and measure $17-25 \times 2.8-3.5 \mu$. Of course this fungus could be an *Ascochyta* phase of *Septoria melicae* Pass. (*S. avenae*), but, in the absence of any evidence that *S. melicae* occurs in the region, it is, perhaps, more conservative to class it with *S. nodorum*.

SEPTORIA CALAMAGROSTIDIS (Lib.) Sacc. on TRisetum SPICATUM (L.) Richt. Material of this species on the leaves of *Trisetum spicatum* from Andrew's Creek, Chelan National Forest, Washington (C. S. 3879) should be mentioned because the fungus shows some distinct differences from material on *Trisetum* spp. from Oregon (20). The spots are white to nearly straw color or sometimes faintly flesh color and have a narrow reddish border. Oregon material showed grayer spots with less distinct borders. The pycnidia in the Chelan material are large, as much as 210μ in diameter, globose to ellipsoid, erumpent, and ostiolate. They are thin-walled, and many of them are eroded or eaten away with only remnants of the bases left. The spores are long, $62-92 \mu$, and wider than any material seen on *Agrostis* or *Calamagrostis*, the narrowest of them measuring 1.7μ wide, about equal to the widest spores seen on *Agrostis* from Oregon. The spores are filiform, with narrowed apices, and slightly swollen, but tapering bases. They range to 2.4μ in diameter. It appears conservative to place this collection in *S. calamagrostidis*. This is the second collection of a filiform-spored *Septoria* on *Trisetum spicatum* made in the state. The other collection, which was obtained on Mt. Rainier, has narrower spores. Possibly it should be compared with *Cylindrosporium calamagrostidis* Ell. and Ev. because the spore sizes are similar.

STAGONOSPORA ARENARIA Sacc. on DACTYLIS GLOMERATA L. In an earlier paper (18) the writer reported this species on *Dactylis* from Ohio and Oregon with one- to three-septate spores, $29-46 \times 3.8-4.6 \mu$. Recent material was collected by George Nyland, Seth Barton Locke, and Avery Rich near Crescent Lake, Washington,

which showed vinaceous, indeterminate lesions bearing globose, scattered, golden-brown pycnidia, 95–160 μ in diameter. The spores were typical of those illustrated earlier but were somewhat larger, 40–52 \times 3.5–5.3 μ , and sometimes five-septate (FIG. 2, *c*). There is some possibility of this fungus finally developing seven-septate spores, which would remove it from *S. arenaria*. This collection, however, must be assigned to *S. arenaria* on the basis of known morphology. In the light of revisions which we have made since our earlier paper appeared, it is considered desirable to mention this specimen and to point out that it can still be assigned to *S. arenaria*. Of the material originally assigned to *S. arenaria*, collections on *Arrhenatherum* (18) have been placed in *Septoria avenae* Frank, and some collections with pointed apices on *Elymus glaucus* (18, FIG. 2, *a*) were assigned to *Septoria agropyrina* Lobik. Collections on *Phalaris arundinacea* L. were found to be immature material of *Stagonospora foliicola* (Bres.) Bubak (20), although in the original condition seen they were excellent facsimiles of *St. arenaria* (compare 17, FIG. 2, *e*, *f* and 20, FIG. 2, *a*).

After excluding the specimens on *Phalaris*, *Scolochloa*, *Arrhenatherum* and a portion of those on *Elymus glaucus*, we have *S. arenaria* listed on the following hosts by states: *Agropyron ciliare* (Trin.) Franch, N. Dak.; *A. cristatum* (L.) Gaertn., N. Dak.; *A. desertorum* (Fisch.) Schult., N. Dak.; *A. repens* (L.) Beauv., Ore.; *A. smithii* Rydb., N. Dak.; *A. spicatum* (Pursh) Scribn. and Sm., N. Dak.; *A. trachycaulum* (Lk.) Malte, N. Dak.; *A. trichophorum* (Lk.) Richt., N. Dak.; *Cinna latifolia* (Trev.) Griseb., Ore., Minn.; *Dactylis glomerata* L., Ore., Wash., Ohio; *Elymus antarcticus* Hook. f., Wash.; *E. canadensis* L., N. Dak.; *E. dahuricus* Turcz., N. Dak.; *E. glaucus* Buckl., Ore., Wash.; *E. junceus* Fisch., N. Dak.; *E. mollis* Trin., Alaska; *E. pseudoagropyron* (Griseb.) Trin., N. Dak.; *E. sibiricus* L., N. Dak.; *E. triticoides* Buckl., Calif.; *Oryzopsis hymenoides* (Roem. and Schult.) Ricker, Mont. A collection on *Puccinellia nuttalliana* (Schult.) Hitchc., from Crystal Springs, N. Dak., is excluded and probably is closer to *Septoria*. It may, perhaps, be undescribed.

PHAEOSEPTORIA FESTUCAE Sprague. Material collected on *Festuca ovina* var. *brachyphylla* (Schult.) Piper at Tioga Pass, Cali-

fornia (C. S. 3561), is referable to *P. festucae*. It should be mentioned because it varies from the type on *F. rubra* L. (19). The spores are light yellow, filiform, and very long, $75-115 \times 2.4-3.4 \mu$ and seven- to eleven-septate. The measurements of the spores in this collection, therefore, mostly range larger than those in the type. The pycnidia are brown, strongly erumpent, more or less gregarious, and $100-160 \mu$ in diameter. The pycnidia, therefore, mostly range in size above the upper limits of the measurements given for this species (19).

Since *P. festucae* and its varieties indicate or possess variable morphology, it is believed logical to include this alpine form in the species. It is associated with *Pyrenophora* sp., *Selenophoma everhartii* (Sacc. and Sydow) Sprague and A. G. Johnson, *Lophodermium arundinaceum* (Schrad.) Chev., *Cladosporium herbarum* Lk. (*Mycosphaerella tulasnei* Jancz.) and traces of *Hendersonia simplex* Schrtr. All of these occur on the dead, overwintered leaves of plants in early head collected June 22, 1947. The plants were growing at an elevation of 10,000 feet at the eastern entrance to Yosemite National Park.

Similar material was found on dead leaves of *Calamagrostis rubescens* Buckl. collected at 5,500 feet elevation above Andrew's Creek, Chelan National Forest, Washington (C. S. 3873). The spores had the typical shape of those of *P. festucae* rather than of the vermisporous ones of *P. calamagrostidis* which occurs on *Calamagrostis* on the seacoast. The spores of the Chelan forest material were $60-98 \times 3.4-4.6 \mu$, light yellow-brown, and seven- to eleven-septate.

CYLINDROSPORIUM CALAMAGROSTIDIS Ell. and Ev. Material of this species on *Muhlenbergia filiformis* (Thurb.) Rydb. was found along Diamond Creek at Palmyra forest camp in the Uinta National Forest, Utah, June 7, 1947 (C. S. 3685). The very small leaves which bore the fruiting bodies of the fungus were yellow, but the lesions themselves were vague brown areas with faintly browner margins. The olive-brown, seriatly arranged acervuli were $80-170 \times 50-80 \mu$. They were erumpent and consisted of truncated, open bodies which scarcely resembled pycnidia as the entire distal third or half of each pycnidium was missing. Dried

spore masses of a resinous, amber color covered the bodies and extended for a short distance beyond their margins. The spores were hyaline, filiform, whip-like (flagelliform), with the basal half very narrowly obclavulate and the apex curving into a very fine tip. The spores were guttulate and questionably one- to three-septate or falsely divided, $50\text{--}72 \times 1.7\text{--}2.5 \mu$ (FIG. 2, f).

C. calamagrostidis was described as having filiform spores, $40\text{--}60 \times 1.5\text{--}2.0 \mu$, borne in acervuli, $150\text{--}200 \mu$. The material on *Muhlenbergia* appears to be morphologically the same as the type on *Calamagrostis*.

SPERMOSPORA SUBULATA (Sprague) Sprague on CALAMAGROS-TIS. Fragmentary material on pinegrass (*Calamagrostis rubescens* Buckl.) was collected on Andrew's Creek, Chelan National Forest, Washington (C. S. 3869), August 11, 1947. The three-septate spores are typical of *S. subulata*, except for size. They measure $50\text{--}80 \times 7.0\text{--}8.4 \mu$. The type on *Melica* has spores only $20\text{--}35 \times 2.5\text{--}4.3 \mu$, but on red fescue and on *Deschampsia* the spores range up to $50 \times 4.6 \mu$. *Calamagrostis* represents an entirely new tribe (*Agrostidae*) for this genus. It has been reported previously on tribes *Festucae* (*Melica* and *Festuca*) and *Avenae* (*Deschampsia*). Since the material on pinegrass consists of only two leaves, it is not possible to study adequately this form with the exceptionally large spores. Because it appears to differ only in relative size (twice as long and twice as wide), it is placed in *S. subulata* (FIG. 2, g). It was associated with undeveloped fruiting bodies of, perhaps, *Mycosphaerella tulasnei*.

It might be mentioned in passing that a spore of a *Spermospora*-like fungus was noted associated with a char spot of *Glyceria pauciflora* Presl. (C. S. 3872). This spore was clear, hyaline, about 80μ long and vaguely resembled a very narrow spore of *Mastigosporium* with a lone apical appendage. This may have been a stray parasite on the char spot. Davis (3) reported *Piricularia parasitica* Ell. and Ev. with spores up to 50μ long and with the apex tapering into a narrow portion, but examination of Davis' collection, sent by H. C. Greene, shows an entirely different fungus.

OVULARIA PUSILLA (Ung.) Sacc. and D. Sacc. [*Ramularia pusilla* Ung., ?*Caeoma pusillum* Bonord., *R. pulchella* Cesati, *O. pulchella* (Ces.) Sacc.].

This fungus is generally known as *O. pulchella*, but it appears that the correct name is *O. pusilla*. The difficulty relative to *O. pusilla* stems partly from an indexing error by Saccardo, who confused *Ovularia haplospora* (Speg.) P. Magn. on *Alchemilla vulgaris* L. with *Ramularia pusilla* Ung. described in 1833 on *Poa nemoralis* L. (24). Lindau (13) pointed this out, and the error is acknowledged by Saccardo (15, v. 18, p. 532). *O. pusilla* is listed by Saccardo and D. Saccardo (15, v. 18, p. 531) as having erect, subramose, caespitose hyphae, arising from stomata and acrogenous, ovate-ellipsoid, non-septate spores. The spore dimensions are lacking but Saccardo states that on checking with figure 10 of Unger's Table II, the dimensions are approximately 8-10 \times 5-6 μ . All evidence indicates that *O. pusilla* is an earlier name than *O. pulchella*, in which case, the latter should be reduced to synonymy.

O. pusilla was collected in Lawyer Canyon State Park, Idaho, June 4, 1947 (C. S. 3641), on an unreported host, *Bromus inermis* Leyss. The spots were small, 1-2 mm. in diameter, mostly circular or sometimes elliptical, gray-buff with a narrow brown border sometimes as broad as the small lighter center. Sometimes ochre or yellow necrotic areas formed around the lesions and were numerous enough to impair photosynthetic activity of the plant. The conidiophores were very obscure and often absent, but in the larger spots they were visible under a low-power binocular as fragile, hyaline structures. A few ovate, crystal-hyaline spores were produced which were 8.5-10.7 \times 5.7-6.8 μ when mature. The fungus is readily referable to *O. pusilla*, and it is mentioned here because of its presence on this important forage grass. Several years ago we found *O. pusilla* on *B. carinatus* near Celilo Falls in Washington. The spots were small and gray. A suitable common name for this disease on *Bromus* spp. would be "round eye spot."

OVULARIA HORDEI (Cav.) Sprague. Cavara (1) reported spores of *Ovularia hordei* measuring only 6-8 \times 4.5 μ on *Hordeum*

but with characteristic, serpentine conidiophores and spores with evident walls. Davis (6), Greene (11), and Sprague (21) report similar material on *Phalaris arundinacea*, Sprague, however, giving larger spore measurements ($11-15.5 \times 6-7 \mu$). Also, *O. baldingeræ* Eliasson has been described on *Phalaris* with spores $9-12 \times 6-7 \mu$, intermediate in length between Cava's measurements and Sprague's. These measurements are very close to *O. pulchella* var. *agropyri* Davis, $9-12 \times 6-9 \mu$ (4). Davis apparently later discarded this variety (7). It appears difficult to segregate *O. hordei* from *O. pusilla* (*pulchella*) on spore size. The tortuous aspect of the conidiophores of *O. hordei* plus the evident spore wall are of diagnostic value. However, material recently collected on *Festuca kingii* in Logan Canyon, Utah (C. S. 3851) shows conidiophores $30-40 \times 3.5 \mu$ with less serpentine traits, some being nearly straight, some moderately twisted. The spores are $16-22 \times 9.5-10.5 \mu$, i.e., larger than *O. hordei* on *Phalaris*. In addition, the faint tendency to muriculations seen in the *Phalaris* material becomes more pronounced in the *Festuca* collections. This brings us to *Ovularia holci-lanati* Cav. (2), which Lindau places in the spiny-spored genus *Ramulaspera* (13) as *R. holci-lanati*. The spores of this species are $16-27 \times 6-10 \mu$ but the conidiophores are given as $170 \times 2 \mu$. These seem abnormally long, but, in the absence of type material, our fescue material cannot logically be assigned to *R. holci-lanati*. In addition, the writer questions if the spores of the fescue collection are spiny enough to be relegated to *Ramulaspera*. It should be added, however, that scattered, yellowish, definitely spiny spores do occur with the minutely spiny, hyaline ones, and only careful culture study can satisfactorily determine this point. It is not impossible that *O. pusilla*, *O. hordei*, *O. lolii* Volk, and *R. holci-lanati* are phases of the same *Ramularia*-like species. At present, it is necessary to recognize all except *O. lolii* Volk, which apparently is the same as the material on *Phalaris* and is assigned to our somewhat emended concept of *O. hordei*. The material on spike fescue is left in *O. hordei*. An English description is appended, but no formal name is given:

Spots burnt umber with a tardily paler, nearly white center. spots elongate-elliptical to linear, $2-4 \times 0.5-1$ mm.; conidiophores few, somewhat arranged in lines, mostly hypophyllous, fascicled and arising from a clump of hyaline cells averaging $14 \times 14 \mu$. several conidiophores from each clump, $30-40 \times 3.5 \mu$, tapering to a sharp apex or somewhat serpentinous in some older stalks, spores ellipsoid-ovate, definitely elongated, usually much narrower at one end, $16-22 \times 9.5-10.5 \mu$, hyaline, with a distinct wall which is minutely echinulate.

LOPHODERMIVM ARUNDINACEUM (Schräd.) Chev. Scattered, large, elliptical perithecia occur as relatively prominent, elongate, black bodies on the white, bleached, overwintered leaves and stems of many alpine and subalpine species of grasses in Utah, Arizona, Washington, and California. The fungus at Tioga Pass, California, at 10,000 feet was sometimes associated with a snow mold, but all evidence indicates that *L. arundinaceum* is a saprophyte. The perithecia ranged up to $700 \times 300 \mu$. The elongated, slit-like ostiole is characteristic. Although the fungus is almost omnipresent on grasses at Tioga Pass, collections were not made on all of them. The following collections or notations were made there and elsewhere.

Agropyron inerme (Scribn. and Sm.) Rydb., Logan Canyon, Utah (C. S. 3657); *Agrostis oregonensis* Vasey, Andrew's Creek, Wash. (C. S. 3873), on old stems mixed with new growth parasitized by *Septogloeum ory-sporum* Sacc., Bomm. and Rouss.; *Calamagrostis breweri* Thurb., Tioga Pass (C. S. 3491, 3567); *Danthonia intermedia* Vasey, Tioga Pass Road (C. S. 3689); *Festuca arizonica* Vasey, Kaibab National Forest, Ariz.; *F. elatior* L., Tioga Pass; *Festuca ovina* L. var. *brachyphylla* (Schult) Piper, Tioga Pass (C. S. 3751); *Muhlenbergia* sp., Mt. Whitney, Calif., at 9,000 and at 9,300 feet (C. S. 3506); *Oryzopsis exigua* Thurb., Chelan National Forest, on Andrew's Creek (C. S. 3698); *Poa compressa* L., Tioga Pass Road; *P. epilys* Scribn., Tioga Pass (C. S. 3574, C. S. 3818); *P. fendleriana* (Steud.) Vasey, Jacob's Lake, Ariz. (C. S. 3550); Bryce National Park, Utah (C. S. 3674); *P. juncifolia* Scribn., Kaibab National Forest, Ariz. (C. S. 3532); *P. leibergii* Scribn., Tioga Pass (C. S. 3568, 3581), Tioga public camp, Calif. (C. S. 3866); *P. longiligula* Scribn. and Wils., Grand Canyon Park, north rim, Ariz. (C. S. 3549); *P. pratensis* L., Tioga Pass Road, McCall, Idaho; *P. secunda* Presl., Bryce National Park (C. S. 3527), Grand Canyon Park, north rim, Ariz. (C. S. 3545); *P. rupicola* Nash, Tioga Pass (C. S. 3572); *Sitanion hystrix* (Nutt.) J. G. Sm., Tioga Pass Road at 8,900 feet (trace only) (C. S. 3578); Grand Canyon, Ariz. (C. S. 3595); *Trisetum spicatum* (L.) Richt., Tioga Pass (C. S. 3579).

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EXPLANATION OF FIGURES.

All illustrations made with the aid of the camera lucida. All drawings $\times 1,000$.

FIG. 1. Pycnospores of fungi on Gramineae. a, From type of *Ascochyta utahensis* on *Agropyron inerme*, Logan Canyon, Utah (C. S. 3610). b, *A. hordei* Hara on *Festuca elatior*, Logan Canyon, Utah (C. S. 3656). c, *A. hordei* on *Hordeum murinum*, Riggins, Idaho (C. S. 3677). d, *A. hordei* on *Bromus carinatus*, Logan, Utah (C. S. 3852). e, *Apiocarpella macrospora* on *Stiporyzopsis bloomeri*, Mt. Whitney, Calif. (C. S. 3605). f, Pycnospores of *Pyrenochaeta* sp., type (C. S. 3631). g, Pycnospores of *Macrophoma sporoboli* on *Sporobolus* sp., Hatch, New Mexico (C. S. 3760). h, Pycnospores of *Macrophoma sporoboli* on *Sporobolus giganteus* near Winslow, Ariz., type (C. S. 3609).

FIG. 2. Spores of fungi on Gramineae. a, *Septoria avenae* on *Festuca elatior*, Logan Canyon, Utah (C. S. 3624). b, *Septoria elymi* on *Agropyron smithii* north of Logan, Utah (C. S. 3468). c, Pycnospores of *Septoria nodorum* on *Festuca subulata* (C. S. 3789). d, Pycnospores of *S. nodorum* on *Elymus glaucus*, Lake Quinault, Wash. (C. S. 3780). e, Pycnospores of *Stagonospora arenaria* on *Dactylis glomerata*, near Crescent Lake, Wash. f, Conidia of *Cylindrosporium calamagrostidis* on *Muhlenbergia filiformis*, Uinta National Forest, Utah (C. S. 3685). g, Conidia of *Spermaspora subulata* on *Calamagrostis rubescens*, Andrew's Creek, Chelan National Forest, Wash. (C. S. 3869).

NOTES ON THE PARASITIC FUNGI OF ILLINOIS¹

LEO R. TEHON

(WITH 15 FIGURES)

In this paper, as in earlier numbers of the series, are described and named as new species a number of plant-inhabiting, presumably parasitic, fungi collected in Illinois. Included also are notes on host range and distribution and critical comments on the differentiation of old species related to those herein described.

Host nomenclature, in the main that of Gray's New Manual of Botany, Seventh Edition, and Bailey's Manual of Cultivated Plants, for wild and cultivated plants respectively, departs in some instances, *e.g.*, *Iris Shrevei* Small, in favor of accepted names.

Cited specimens are designated by places and dates of collection and their accession numbers in the Mycological Collection of the Illinois State Natural History Survey, in which they are deposited together with the original or representative microscopical slides upon which these studies were developed.

Leptothyrium anthelmintici sp. nov.

Maculis in caulibus hospitis effusis, non limitatis; pycnidiis dimidiatis, rotundatis, bene fornicatis, nigris, nitidis, subcuticularibus, contextu pseudoparenchymato non radiante, saepe fimbriatis hyphis brunneis subcuticularibus, 125–150 μ diametris, poro centrali rotundo 10–15 μ lato apertis; sporis hyalinis, continuis, oblongis vel pediformibus, superne rotundatis, basim leniter attenuatis, 18–23 \times 5–7 μ .

Caulicolous, in extensive, unlimited pale tan areas; pycnidia dimidiate, round, well arched, black, shining, subcuticular, pseudoparenchymatous, not radiate, often fimbriate with few to many brown, long, subcuticular hyphae, 125–150 μ in diameter, opening by a central, round pore 10–15 μ wide; spores hyaline, nonseptate, oblong or pediform, the distal end bluntly rounded, the basal end somewhat tapered, 18–23 \times 5–7 μ .

¹ Earlier *Notes* in this series appear in *Mycologia* as follows: 16: 135–142. 1924; 17: 240–249. 1925; 19: 110–129. 1927; 21: 180–196. 1929; 25: 237–257. 1933; 29: 434–446. 1937.

On dead stems of *Chenopodium ambrosioides anthelminticum* (L.) Gray, Harrisburg, Saline County, Ill., August 16, 1943, Acc. No. 30049 (type).

Although also a stem inhabitant, this second species of *Leptothyrium* to be recorded on *Chenopodium* differs from *L. Chenopodii* Dearn. in that its pycnidia are somewhat smaller while its spores, differently shaped, are three to five times as long.

Leptothyrium Avenae sp. nov.

Pycnidii sub stomatibus in glumis et pedi hospitis seriatis sitis, subcuticularibus, nigris, immersis, cum cellis externis aliformibus et radiatis, plerumque late ellipticis, $100-200 \times 65-140 \mu$; poro minuto sub stomati apertis; sporis hyalinis, continuis, ovalibus, oblongis vel pediformibus, $4-8.5 \times 16-23 \mu$; sporophoris simplicibus, brevibus, acutis.

Pycnidia substomatal, in rows on the outer faces of glumes and on pedicels, subcuticular, black, remaining immersed, with cells of the outer wall aliform and radiate, for the most part broadly elliptical, sometimes nearly round, $100-200 \times 65-140 \mu$; pore minute, substomatal; spores hyaline, nonseptate, oval, oblong or pediform, $4-8.5 \times 16-23 \mu$; sporophores simple, short, acute.

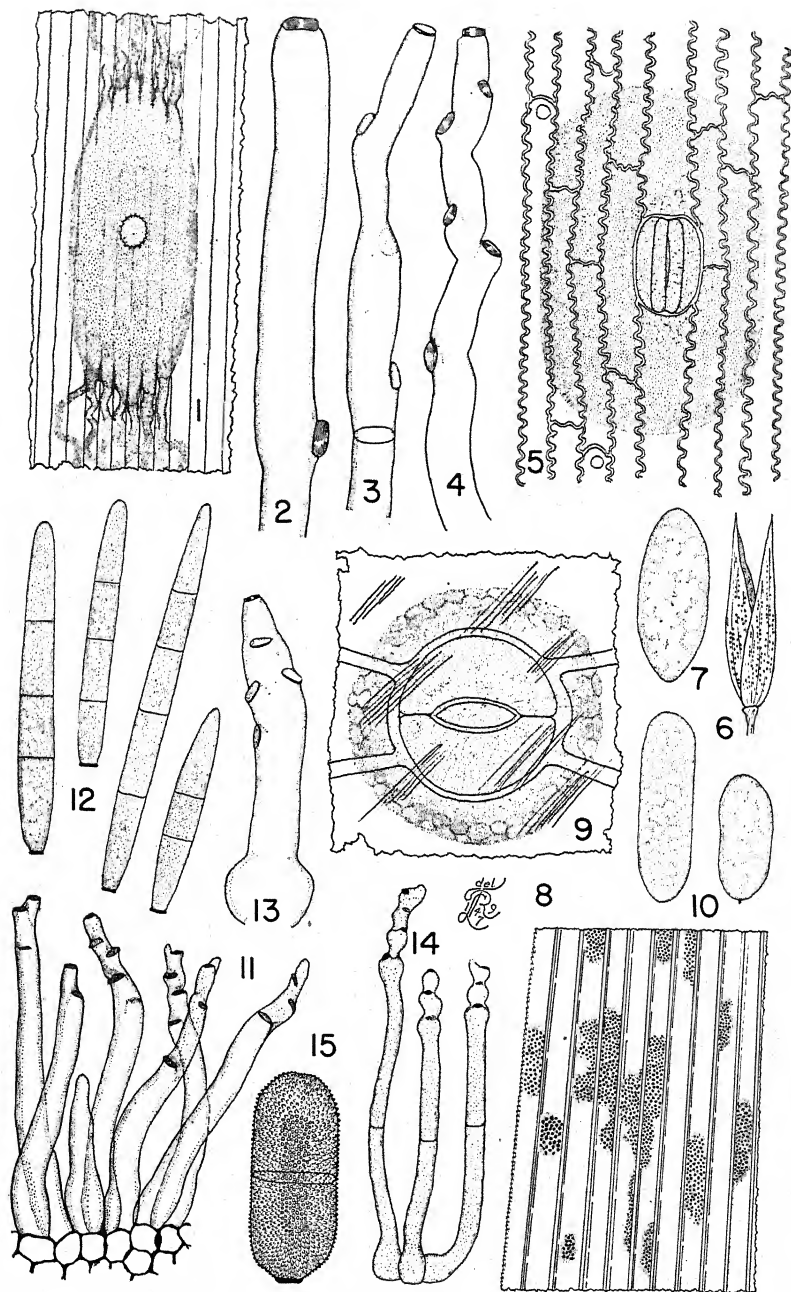
On *Avena sativa* L., Woodford, Woodford County, Ill., July 1, 1938, Acc. No. 29926 (type). (FIGS. 5-7.)

Oat spikelets, when attacked on the glumes and pedicels by this fungus, develop poorly and bear small grains. Also, their pedicels are short and spread less widely than normal from the axis of the panicle. The association of pycnidia with stomata appears constant; hence, the arrangement of stomata in rows along each side of the veins determines the serial arrangement of the pycnidia.

From the imperfectly described *Leptostroma Avenae* Brun., this fungus appears well distinguished by its lack of a longitudinal slit and by its longer, wider spores.

Leptothyrium fimbriatum sp. nov.

Maculis amphigenis, 1-12 mm. diametris, rotundatis, atrocinereo-griseis, zona angusta brunnea circumdatis; pycnothyriis plerumque epiphyllis, subcuticularibus, nigris, nitidis, $60-150 \mu$ diametris, saepe ambitu fimbriatis et hyphis aliformibus subcuticularibus connexis; centro poro rotundo, $6.5-13 \mu$ diametro apertis; sporis ovalibus usque sphaericis, hyalinis, continuis, $6.5-12 \times 8-13.5 \mu$.



FIGS. 1-15. Characteristics of Illinois fungi.

Spots amphigenous, 1–12 mm. in diameter, round, dark ash-gray, narrowly brown-bordered; pycnothyria for the most part epiphyllous, occasionally hypophyllous, subcuticular, round, black, shining, 60–150 μ in diameter, appearing parenchymatic but their outermost layer consisting of aliform cells, often more or less fimbriate at the margins and frequently connected by proliferating, subcuticular strands of aliform mycelium; pore present, central, 6.5–13 μ in diameter; spores oval to spherical, hyaline, nonseptate, 8–12 \times 6.5–13.5 μ .

On leaves of *Smilax hispida* Muhl., Topeka, Mason County, Ill., October 20, 1937, Acc. No. 30050 (type); on leaves of *Smilax rotundifolia* L., Sparta, Randolph County, Ill., July 28, 1926, Acc. No. 20945.

In 1933 the writer (9) called attention to the second collection cited above, assigning it tentatively to *Phyllosticta Smilacis* Dearn., and in figure 7 illustrated the fringe of aliform mycelium often found surrounding the margins of the pycnothyria. In the first collection cited above, which contains the more abundant material, is obviously the more mature, and, therefore, is the better suited for specification as the type specimen, the marginal aliform mycelium often extends, at irregular points, as simple or branched, subcuticular, flat strands of aliform hyphae that stretch from one pycnothyrium to another. As these strands never appear to meet between pycnothyria, it seems clear that pycnothyria develop upon them at intervals as they grow in length.

This species, being foliicolous, is not likely to be confused with *Leptothyrium Smilacis* Dearn., which, as Dearness (1) recorded, is caulicolous and has oblong pycnidia up to 2 mm. long and oblong spores measuring 6–12 \times 3 μ .

***Leptostromella Solani* sp. nov.**

Pycnidiis in caulibus in maculis brunneolis 1–5 mm. longis, dimidiatis, nigris, nitidis, subcuticularibus, anguste ellipticis, 300 μ vel ultra longis, 120–150 μ latis; mycelio clypei aliformi; loculo 120–150 \times 90–110 μ ; poro centro rotundo apertis, postea longitudinaliter disruptis; sporis acicularibus usque fusiformibus, continuis, hyalinis, utrinque acutis, rectis curvulisve, 11–19 \times 1.5 μ .

Caulicolous in pale brown areas surrounding stems, extending 1–5 cm. in length, and limited by narrow, dark brown, slightly raised margins; pycnidia dimidiate, black, shining, subcuticular,

narrowly elliptical, $300\ \mu$ or more long, $120\text{--}150\ \mu$ wide; mycelium of the clypeus aliform; locule $120\text{--}150 \times 90\text{--}110\ \mu$; opening at first by a central, round pore, later by a lengthwise split; spores acicular to spindle-shaped, nonseptate, hyaline, acute at both ends, straight or variously curved, $11\text{--}19 \times 1.5\ \mu$.

On stems of *Solanum carolinense* L., LeRoy, McLean County, Ill., October 11, 1943, Acc. No. 30051 (type). (FIG. 1.)

Although the outermost layer (called clypeus in the above description) of the pycnidium is composed of cells clearly recognizable as of aliform origin, the ends of the pycnidium usually have extending from them several subcuticular hyphae, dark brown near the pycnidium but fading to hyaline at a distance from the pycnidium, that show no aliform branching. Evidence of the radiate character of the pycnidium is to be found, therefore, only in the cells of the clypeus.

ACTINOPELTE DRYINA (Sacc.) Hoehn.

Limber and Cash (3) have carefully considered the morphology, taxonomy and synonymy of this fungus and have given an extended and detailed description of it as they observed it on a variety of hosts. They did not, however, attempt to obtain for examination three species described by the writer, although they listed these species as questionable synonyms. The names of these species, published as *Actinothyrium gloeosporioides* Tehon (8), *Pirostoma Nyssae* Tehon (8) and *Leptothyriella Liquidambaris* Tehon & Stout (10), each with accompanying figures, are of course to be referred to *Actinopelte* and, considering the broad inclusiveness of Limber and Cash's specific description, to the species *dryina* as well. Hence, the occurrence of *Actinopelte dryina* on *Liquidambar styraciflua* in Illinois as well as Maryland and on *Sassafras officinale* in Illinois as well as New Jersey may be recorded. Although in the Illinois samples on *Nyssa sylvatica* "mature" spores are brown—deeper in color than olivaceous and without a green tint—the same color may be found in "mature" spores on *Quercus* and other hosts. The fine verrucose markings appear clearly only on the more "mature" spores. Hence, there can be little doubt that the Illinois material on *Nyssa* is conspecific with material collected on the same host by Diehl in Virginia, which Limber and Cash examined.

Additional records, place and host, for Illinois are as follows:

On Acer saccharum Marsh.—Buncombe, Johnson County, August 5, 1922 (Acc. No. 29732) and Kinderhook, Pike County, June 28, 1944 (Acc. No. 30074). In these samples, a large majority of the fruiting bodies are on the upper leaf surface, toward the periphery of circular spots 8–10 mm. in diameter that are dotted below with *Gloeosporium acervuli*. This habit is like that on *Sassafras*, where the rhizothyrria are found epiphyllous on spots sometimes carrying hypophyllously the acervuli usually called *Gloeosporium affine*. The rhizothyrria are small, mostly 60 to 65 μ in diameter, but the spores are of the usual size, measuring 11–13 \times 8–9.5 μ . On the same host are specimens taken at Bald Knob, Union County, June 13, 1927 (Acc. No. 29734), at Carbondale, Jackson County, July 29, 1938 (Acc. No. 29733), at White City, Macoupin County, August 30, 1937 (Acc. No. 29742), and at Ozark, Johnson County, July 18, 1931 (Acc. No. 29747).

On Cercis canadensis L.—Mounds, Pulaski County, August 10, 1926 (Acc. No. 29737). On this host the spots range from 3 to 5 mm. in diameter, are round or somewhat irregular, light brown with narrow purple brown borders, and obviously had their origins in minute hypophyllous galls resulting from the egg-laying activities of an insect. On this host, as on *Acer saccharum*, *Actinopelte dryina* appears to be a secondary invader.

On Fraxinus americana L.—Dongola, Union County, August 10, 1929 (Acc. No. 29736); Boskydell, Jackson County, July 23, 1923 (Acc. No. 5562); Eagle Mountain, Saline County, July 14, 1922 (Acc. No. 29741); and Balcom, Union County, August 11, 1934 (Acc. No. 29744). Spots range from 4 to 15 mm. in diameter, are tan with narrow purple borders above and tan without borders below. On these samples, rhizothyrria are exceptionally abundant and occur on both surfaces of most spots, epiphyllously only in a few spots.

On Fraxinus tomentosa Michx. f.—Dongola, Union County, August 11, 1927 (Acc. No. 29738).

On Sassafras albidum (Nutt.) Nees.—Fountain Bluff, Jackson County, June 20, 1924 (Acc. No. 17547); on var. *molle* (Raf.) Fern.—Marlow, Jefferson County, September 7, 1926 (Acc. No. 29739). In this sample the spots bear no acervuli of *Gloeosporium* on the underside but, like the sample on *Cercis canadensis*, contain small, centrally located insect galls. The rhizothyrria are exclusively epiphyllous. Also at Salem, Marion County, August 13, 1926 (Acc. No. 29745), and Mounds, Pulaski County, August 10, 1926 (Acc. No. 29748).

On Ulmus alata Michx.—Sanburn, Johnson County, June 15, 1927 (Acc. No. 29740). Spots are darker brown on this host than on most others and small, reaching 3 to 4 mm. in diameter. The rhizothyrria are more numerous on the upper, but frequent also on the lower, surfaces of the spots.

On Liquidambar styraciflua L.—Elizabethtown, Hardin County, June 17, 1927 (Acc. No. 29743).

On Carya ovata (Mill.) K. Koch.—Simpson, Johnson County, July 11, 1929 (Acc. No. 29746).

On Quercus as follows: On unidentifiable *Quercus* seedling leaves at Mounds, Pulaski County, August 10, 1926 (Acc. No. 29749); on *Quercus*, leaf fragments not identifiable to species, in Pope County, August 3, 1922

(Acc. No. 11752), and Dongola, Union County, August 12, 1922 (Acc. No. 995); on *Q. imbricaria* Michx. at West Union, Clark County, September 27, 1930 (Acc. No. 29750), Salem, Marion County, August 13, 1926 (Acc. No. 29751), Kimmundy, Marion County, June 14, 1922 (Acc. No. 29752), Red Bud, Randolph County, August 24, 1926 (Acc. No. 27753), Brownsville, White County, July 11, 1922 (Acc. No. 5164), and Parker, Johnson County, July 27, 1922 (Acc. No. 1880). On this host rhizothyrria may be found in spots ranging from 3 mm. in diameter to one third or more of the size of the leaf and are as abundant on the lower sides as on the upper sides of the spots. On *Q. marilandica* Muench. at Pinckneyville, Perry County, October 27, 1927 (Acc. No. 29754), Flora, Clay County, July 23, 1927 (Acc. No. 29755), and Edwardsville, Madison County, June 15, 1944 (Acc. No. 30075); on *Q. palustris* Muench. at West Union, Clark County, September 27, 1930 (Acc. No. 29735), Reevesville, Johnson County, July 27, 1922 (Acc. No. 29756), Makanda, Jackson County, August 19, 1922 (Acc. No. 29757), Pulaski County, August 9, 1922 (Acc. No. 8553), Reevesville, Johnson County, July 27, 1922 (Acc. No. 1833), and Hagerstown, Fayette County, September 1, 1944 (Acc. No. 30127); on *Q. stellata* Wang. at Lusk, Pope County, June 16, 1927 (Acc. No. 29758), and Golconda, Pope County, July 28, 1922 (Acc. No. 1000); on *Q. velutina* Lam. at Boskydell, Jackson County, July 23, 1923 (Acc. No. 6423), Renault, Monroe County, July 20, 1923 (Acc. No. 10221), and Fountain Bluff, Jackson County, June 20, 1924 (Acc. No. 16125); *Q. borealis maxima* (Marsh.) Ashe at Fountain Bluff, Jackson County, June 20, 1924 (Acc. No. 5541), Goreville, Johnson County, June 22, 1924 (Acc. No. 3223), Makanda, Jackson County, August 18, 1922 (Acc. No. 11134), Tunnel Hill, Johnson County, July 25, 1922 (Acc. No. 2925), and Seymour, Champaign County, October 15, 1925 (Acc. No. 9282); on *Q. alba* L. at Albion, Edwards County, June 13, 1944 (Acc. No. 30073).

On *Rhus Toxicodendron* L.—At Sandoval, Marion County, June 6, 1944 (Acc. No. 30072). On the one sample rhizothyrria occur epiphyllously only in spots associated closely with spots containing pycnidia of *Phyllosticta rhoicola* Ell. & Everh. As the two were not observed in the same spot, it is not clear that the *Actinopelte* follows the *Phyllosticta*.

Stictopatella Iridis sp. nov.

Maculis atro-griseis, nigrescentibus, nervis foliorum limitatis, 1 mm. usque 1 cm. longis, subinde confluentibus; pycnidiiis numerosis, amphigenis, patelliformibus, rotundatis usque late ellipticis, 55–90 × 60–125 μ , sub stomatibus sitis, patelliformibus; sporophoris numerosis, hyalinis, acicularibus, apice acutis, 1.5 μ latis, usque 15 μ longis, simplicibus; sporulis hyalinis, continuis, oblongis, utrinque rotundatis, 5.5–10 × 2.2–3.3 μ .

Spots dark gray, becoming black, on one or both leaf surfaces, limited laterally by veins, 1 mm. to 1 cm. long or much longer by confluence; pycnidia numerous, amphigenous, cup-shaped, circular to broadly elliptical, 55–90 × 60–125 μ , located beneath stomata, often contiguous but remaining distinct, cup-shaped to patelliform, without a cover, brown, pseudoparenchymatous; sporophores very

numerous, hyaline, slender, pointed, $1.5\ \mu$ wide, up to $15\ \mu$ long, simple; spores hyaline, nonseptate, oblong, ends rounded, $5.5\text{--}10 \times 2.2\text{--}3.3\ \mu$.

On leaves of *Iris Shrevei* Small, Urbana, Champaign County, Ill., June 11, 1947 (com. R. A. Evers), Acc. No. 30048 (type). (FIGS. 8-10.)

Casual examination of this fungus might result in its placement in *Gloeosporium*. It is, however, truly patelliform, having a distinct but shallow pseudoparenchymatous cup two or three cells thick within which conidiophores arise as in a pycnidium. Apparently no cover is ever developed by the pycnidium, which invariably develops in a substomatal cavity. The long, slender sporophores converge from the inner wall of the cup toward the stomatal opening, through which the spores, cut off one at a time from the tips of the conidiophores, escape.

As a result of reexamination of *Phyllosticta destructiva* var. *Evonymi* Desm., von Hoehnel (2, p. 166) proposed the genus *Stictopatella*, with *S. Evonymi* (Desm.) v. Hoeh. as the first species. His placement and characterization of this genus, translated, was as follows:

Patelloideae-patellatae: pycnidia disc shaped, developed beneath the epidermis, more or less erumpent; basal layer thin, indefinitely cellular; cover thin, consisting of fine, slender cells; conidiophores basal, simple, crowded, parallel; conidia terminal, one-celled, hyaline, rotund or elongate; leafspot inhabitant.

Although our iris-inhabiting fungus lacks a pycnidial cover and does not become erumpent, it appears in all other respects so closely congeneric with Hoehnel's *Stictopatella* as to merit assignment in that genus.

***Asperisporium Acori* sp. nov.**

In foliis emortuis et marginibus emortuis foliorum vivorum; conidiophoris erectis, simplicibus, solitariis seu plerumque 2 usque 30 in caespitibus congestis laxis emergentibus per stomates, brunneis, eseptatis seu quoque septimentis uno medio vel infra locato divisio, basim nonnihil inflatis sed raro bullatis, levibus, diametro similibus, at apicem initio rotundatis demum contortis et nodulosis, cum uno minus saepe duobus hilis minutis, fuscis, protrudentibus ad apicem et quemque nodum, $4\text{--}8\ \mu$ crassis, $30\ \mu$ (conidium primum gignentibus) usque $120\ \mu$ longis; conidiis acrogenis, plerumque non

sed interdum catenulatis, echinulatis, brunneis, typice 1-septatis, non constrictis, seu quoque eseptatis vel 2- et 3-septatis, oblongis, utrinque rotundatis, basim hilo conspicuo fusco protrudenti ornatis, typice $21 \times 9.5 \mu$ seu quoque ab $14 \times 5.5 \mu$ usque $30 \times 13.5 \mu$ an continuis, 2-, vel 3-septatis.

Inhabiting dead leaves and dead tissue on the margins of living leaves; conidiophores erect, simple, single or for the most part 2 to 30 or more crowded in loose fascicles that emerge through stomata, light brown, nonseptate or with one septum located at about the middle or below, at the base somewhat but not usually bulbously enlarged, smooth, of quite uniform diameter, at the tip bluntly rounded at first but becoming contorted and nodulose following production of successive spores, with one, less often two, small, dark, protruding geniscars at the apex and on each of the nodal shoulders, $4-8 \mu$ wide, from 30μ (when the first spore may be borne) to 120μ long; spores acrogenous, not usually but occasionally catenate, conspicuously echinulate, brown and typically of the same tint as the conidiophores, typically one-septate but also non-septate and with two and three septa, not constricted at the septa, oblong, with rounded ends and at the base a conspicuous, dark, protruding geniscar, typically $21 \times 9.5 \mu$ but ranging from $14 \times 5.5 \mu$ to $30 \times 13.5 \mu$ for 3- and 4-celled spores.

On *Acorus Calamus* L., Urbana, Champaign County, Ill., June 17, 1926, Acc. No. 30124 (type). (FIGS. 14, 15.)

The genus *Asperisporium*, established by Maublanc (4, 5), is based, as to type species, on *A. Caricae* Maubl. (*Cercospora Caricae* Speg., *Scolecotrichum Caricae* Ell. & Everh., *Epiclinum Cumminsii* Massee, *Pucciniopsis Caricae* Earle) and, as to accompanying species, on the morphological agreement of *Fusicladium Peucedani* Ell. & Holw., *Scolecotrichum Alatroemeriae* Allesch., and *S. punctulatum* Tracy & Earle with the fungus on *Carica*, all of which Maublanc transferred to *Asperisporium*.

Upon comparison with the original description and illustrations, the Illinois fungus on *Acorus* proves congeneric with Maublanc's *Carica* fungus but differs specifically in lacking a highly developed stroma, in having more slender, longer, spreading conidiophores and generally larger spores densely covered with minute, conical echinulations rather than irregular, thick, platelike verrucae.

Cercospora difformis sp. nov.

Maculis rotundatis, 0.5-2 mm. diametris, albis, zona angusta, lutea elevata definitis, demum deicientibus; caespitulis amphigenis sed plerique epiphyllis,

tuberculis parvis, raris, cellarum brunnearum compositis oriundis, unum conidiophorum usque 20, plerumque 12 vel 14, continentibus, cum conidiophoris externis communiter procumbentibus et eis internis rigide erectis et unam densam fasciam vel duas formantibus; conidiophoris brunneis, difformibus; eis procumbentibus in partes tres, basilare 20–30 μ longa, 3 vel 4 cicatrices sporarum gerente, mediata nonnumquam 100 μ longa cicatricem nullam gerente, et distante usque 125 μ longa, 1 usque 8 cicatrices gerente, tota conidiophora parte distante excepta regulariter intervalibus 20–26 μ septata, usque 320 μ longa, 4–5.5 μ crassa; eis erectis 1- vel 2-septatis prope basim et unam cicatricem sporae usque 5 gerentibus, 45–110 μ longis, 4.5–6 μ crassis, minus tenuibus quam quae sunt procumbentes; sporis hyalinis, acicularibus, base ad apicem pariter tenuatim, prope basim intervalibus 10–12 μ septatis, ultra septis imperspicuis, 80–250 μ longis, 2.5–4 μ crassis.

Spots round, 0.5 to 2 mm. in diameter, white, with narrow, raised, yellow to tan borders, eventually falling out; caespitules amphigenous but more numerous on the upper surface, arising from small, loose tubercles of brown cells, consisting of 1 to 20, commonly 12 to 14, conidiophores, the outer conidiophores usually procumbent, the inner ones standing stiffly erect in one or two tight fascicles; conidiophores brown, of two types, a procumbent kind consisting of a basal section 20 to 30 μ long with 3 or 4 spore scars, a central section sometimes 100 μ long without spore scars, and a distal section up to 125 μ long bearing one to 8 spore scars, the whole conidiophore regularly septate at intervals of 20 to 26 μ except in the terminal section, up to 320 μ long, 4–5.5 μ wide, and an upright kind with one, sometimes two, septa very near the base and one to 5 spore scars, 45–110 μ long, 4.5–6 μ wide, less slender than the procumbent kind; spores hyaline, acicular, tapering regularly from base to tip, septate in the basal part at intervals of 10 to 12 μ , septa in the fine tip indistinct, 80–250 μ long, 2.5–4 μ wide.

On leaves of *Viola* (*papilionacea* Pursh?), *Vandalia*, Fayette County, Ill., October 11, 1944, Acc. No. 30224 (**type**); Abingdon, Knox County, Ill., August 7, 1922, Acc. No. 16200. On *V. sororia* Willd., Roseville, Warren County, Ill., July 31, 1922, Acc. No. 15567. (FIG. 3.)

Cercospora sororiae sp. nov.

Maculis majusculis, rotundatis usque ovalibus, 1 cm. vel plus latis aut non limitatis et partem magnam folii destruentibus, supra luteo-brunneis, subtus brunneis; caespitulis numerosissimis, amphigenis sed abundantioribus subtus, e paucis usque 100 sporophoribus efformatis, e tuberculis nigris, densis, stromatoideis plerumque 30–50 μ latis exsurgentibus; conidiophoris pallide brunneis, confertis, erectis, sursum effusis et cum aliquot prominentibus congestis

cicatricibus sporarum, non septatis, non ramulosis, $3-4 \times 25-40 \mu$; sporis hyalinis usque pellucidis fumoso-brunneis, teretibus, apice rotundatis, base cicatricibus externis conspicuis fuscis ornatis, 2- usque 5- plerumque 3-septatis, $3.5-4 \times 30-60 \mu$.

Spots rather large, round to oval, 1 cm. or more wide or unlimited and involving a large part of the leaf, yellow brown above, brown below; cespitules very numerous, amphigenous but much more abundant on the lower leaf surface, consisting of few to 100 or more sporophores rising from black, dense, stroma-like tubercles usually 30 to 50μ wide; conidiophores pale brown, crowded, erect, spreading above and with several prominent spore scars crowded at the tips, nonseptate, simple, $3-4 \times 25-40 \mu$; spores hyaline to smoky brown, terete, rounded at the apex, bearing at the base conspicuous dark, external scars, two- to five- but for the most part three-septate, $3.5-4 \times 30-60 \mu$.

On leaves of *Viola sororia* Willd., Kinderhook, Pike County, Ill., June 28, 1944, Acc. No. 30126 (type). (FIGS. 11, 12.)

The addition, above, of two species of *Cercospora* to the group of eight already described is justifiable on the ground that the new species are distinct and readily separable. Among the older species *C. macrospora* Osterw. is distinguished by the swordlike appendage at the base of the spore, a character aligning it as one of the synonyms of *Centrospora acerina* (Hartig) Newhall (6). On the basis of extant descriptions, it and the other species can be rather clearly sorted under the system proposed by Solheim (7), as follows:

- I. Each spore bearing a slender, retrorsely directed appendage at its base (*Cercospora macrospora* Osterw.)...*Centrospora acerina* (Hartig) Newh.
- II. Spores without such appendages. *Cercospora*.
 - A. Only internal mycelium present.
 1. Conidiophores simple.
 - a. Stroma tuberculate, spores cylindrical (Solheim's Section III); conidiophores short, nonseptate (FIG. 11); spots unlimited*C. sororiae*
 - a. Stroma loose or absent.
 - b. Spores acicular-obclavate (Solheim's Section IV).
 - c. Conidiophores all more or less erect and alike.
 - d. Conidiophores short; spore scars several, often causing geniculations (FIG. 4) ...*C. Violae* Sacc.
 - d. Conidiophores long, expanded toward the tips; spore scars few, widely separated (FIG. 2)
C. Violae-tricoloris Br. & Cav.

- c. Conidiophores of 2 kinds, procumbent and erect: the procumbent very long, septate, with spore scars at base and tip; the erect shorter, closely fascicled, with spore scars at tip *C. difformis*
- b. Spores cylindrical (Solheim's Section VI).
 - e. Conidiophores repent, very long, hypophyllous; spores 5-6.5 μ wide *C. lilacina* Bres.
 - e. Conidiophores erect, shorter, amphigenous; spores more slender, up to 4.5 μ wide.
 - f. Conidiophores very short, nonseptate, with spore scars crowded near the tips (FIG. 13); spots unlimited *C. granuliformis* Ell. & Holw.
 - f. Conidiophores longer, septate; spots circular, limited *C. Violae-sylvaticae* Oud.
- 2. Conidiophores alternately branched, stroma loose, spores cylindrical (Solheim's Section X) *C. murina* Ell. & Kellerm.
- B. Both external and internal mycelium present, conidiophores simple, stroma loose, spores abruptly obclavate (Solheim's Section XVI); spots small, limited *C. li* Trail.

Of the old species listed in the above key, we have specimens that may be assorted as given below:

C. granuliformis Ell. & Holw. on *Viola (papilionacea* Pursh?): America, Pulaski County, June 18, 1941, Acc. No. 28309; Bement, Piatt County, July 6, 1925, Acc. No. 1576; Marshall, Clark County, July 22, 1941, Acc. No. 28336.—This species is readily recognized by the extensive leaf area which it kills, its small, compact cespitules, the notable distribution of cespitules along the leaf veins, and its very short conidiophores which are bulbous at the base and bear one to several closely crowded spore scars toward the tip (FIG. 13). It and the new *C. sororiae* described above are very similar, differing chiefly in size and compactness of stroma and conidiophores (FIGS. 11, 12).

C. Violae Sacc. on *Viola (papilionacea* Pursh?): Bement, Piatt County, July 6, 1925, Acc. No. 4087; Clinton, DeWitt County, July 21, 1925, Acc. No. 11986; Kansas, Edgar County, July 17, 1925, Acc. No. 3952; Mackinaw, Tazewell County, June 6, 1923, Acc. No. 17411; Mahomet, Champaign County, August 30, 1921, Acc. No. 10385; Mason, Effingham County, June 11, 1936, Acc. No. 25484; Oneida, Knox County, August 9, 1922, Acc. No. 13811; Peonydale Farm, McDonough County, July 20, 1922, Acc. No. 15775; Pittsfield, Pike County, July 24, 1925, Acc. No. 8676.

On *Viola sororia* Willd.: Lewistown, Fulton County, July 12, 1922, Acc. No. 16312; Thompsonville, Franklin County, July 21, 1922, Acc. No. 2705.—Characterized by small, round, persistent spots usually 1–2 mm. in diameter, acicular spores, virtual absence of a tubercle, and short conidiophores markedly geniculate and with several spore scars near the tip (FIG. 4), *C. Violae* is apparently the most common species on *Viola* in Illinois. Although Saccardo's description gives the spore length as 150–200 μ , spores 150 μ long are uncommon in Illinois samples; the usual spore lengths range between 55 and 135 μ .

C. Violae-tricoloris Br. & Cav. on *Viola (missouriensis* Greene?): Lawrenceville, Lawrence County, June 9, 1942, Acc. No. 28687.—The single specimen referred to this species is so placed with hesitation. Although the spots, 3 to 4 mm. in diameter, are larger than those of other "round spot" species and the conidiophores and spores agree well as to measurements with the description, the conidiophore apex is distinctive in appearance (FIG. 2). When the first spore is seen on the tip of the conidiophore, the tip appears swollen or inflated. After discharge of the spore, growth of the conidiophore continues at this larger diameter, and spore scars, widely spaced, become prominent, dark excrescences on the side of the hypha, causing no indentations or geniculations.

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EXPLANATION OF FIGURES

FIG. 1. *Leptostromella Solani*, surface view of a pycnidium. FIG. 2. *Cercospora Violae-tricoloris*, terminal part of a conidiophore. FIG. 3. *Cercospora difformis*, terminal part of a procumbent conidiophore. FIG. 4. *Cercospora Violae*, terminal part of a conidiophore. FIGS. 5-7. *Leptothyrium Avenae*: 5, surface view of a pycnidium centered beneath a stoma; 6, habitat sketch; 7, a spore. FIGS. 8-10. *Stictopateella Iridis*: 8, habitat sketch; 9, pycnidium beneath a stoma; 10, two spores. FIGS. 11-12. *Cercospora sororiae*: 11, group of conidiophores; 12, four spores of different sizes. FIG. 13. *Cercospora granuliformis*, conidiophore. FIGS. 14-15. *Asperisporium Acori*: 14, three conidiophores; 15, a spore.

KERATINOPHILIC CHYTRIDS. III. RHIZOPHYDIUM NODULOSUM SP. NOV.

JOHN S. KARLING¹

(WITH 20 FIGURES)

In previous publications the author (3, 5) reported on the use of keratinized tissues such as hair, skin, hoofs, horns, nails and feathers as substrata for isolating specialized types of chytrids, and described two new species which appear to be limited in occurrence to tissues containing keratin. The present contribution deals with a third new species isolated on human hair from muck soil from Van Cortlandt Park, New York City, and grown on other keratinized substrata in filtered, sterile soil extract. This species is characterized by ectobiotic sporangia and branched endobiotic rhizoids which distinguish it as a species of *Rhizophyidium*. More specifically it is distinguishable by sporangia with a large number of prominent exit papillae which give them a strikingly gibbose, angular or nodular appearance and shape. On the basis of this and other structural characteristics as well as the fact that it grows only on keratinized substrata in nature, it is distinct from the other known species of this genus. Accordingly, it is diagnosed as a new species and named *R. nodulosum* because of the characteristic shape and appearance of the sporangia.

Rhizophyidium nodulosum sp. nov.

Fungus saprophyticus. Sporangii hyalinis, laevibus, plerumque angularibus, irregularibus, $65\ \mu$ diam., aut ovalibus, $10-25 \times 15-50\ \mu$ diam., aut sphaericis, $10-35\ \mu$, oblongis vel pyriformibus, $1-15$ papillatis, $4-8 \times 8-15\ \mu$ diam. Zoosporis sphaericis, $2.8-3.2\ \mu$ diam., globulo refractivo $0.4-0.8\ \mu$ diam., flagello $15-18\ \mu$ longo. Hyphis rhizomorphis ramosis, $30-270\ \mu$ longis. Sporibus perdurantibus ignotis.

Sporangia hyaline, smooth, predominantly angular or nodular, up to $65\ \mu$ in diam., oval, $10-25 \times 15-50\ \mu$, spherical, $10-35\ \mu$, oblong, pyriform or irregular with $1-15$ prominent exit papillae,

¹ The author is very grateful to T. K. Just of the Chicago Natural History Museum for the Latin diagnoses.

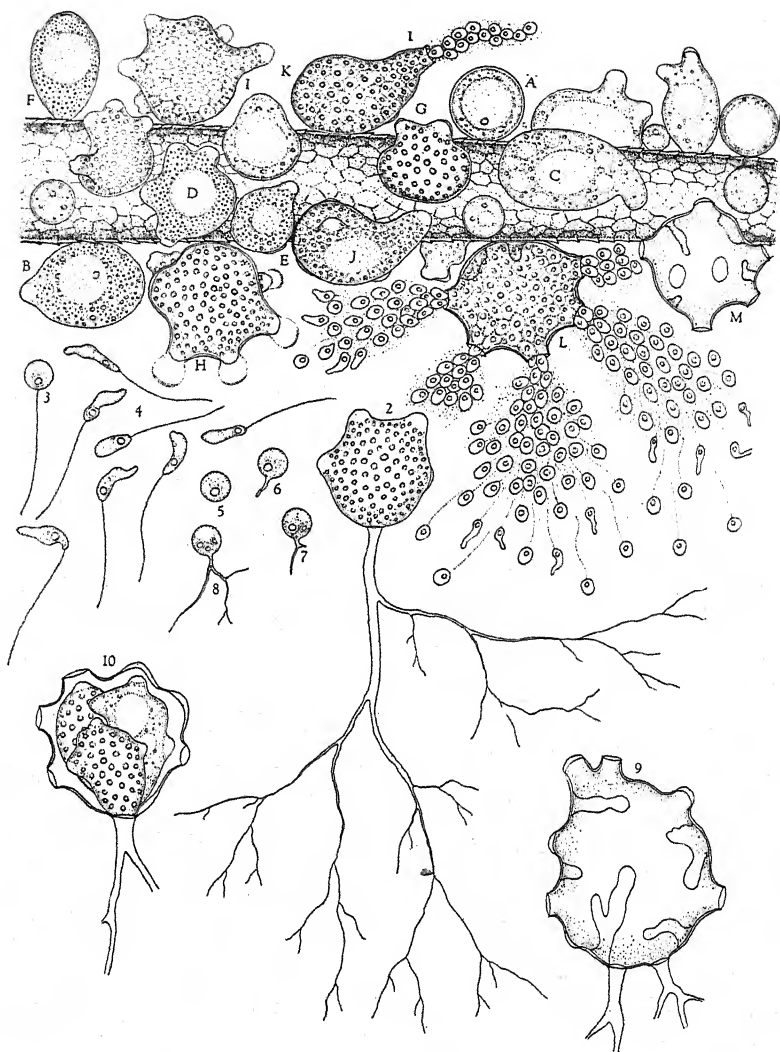
4-8 μ high by 8-15 μ broad at the base. Zoospores spherical, 2.8-3.2 μ , with a minute refractive globule, 0.4-0.8 μ diam.; flagellum 15-18 μ long. Rhizoids usually well developed but sparingly branched, arising from one to several points on the base of the sporangium, main axes up to 7 μ in diam., branches extending for distances of 30-270 μ . Resting spores unknown.

Saprophytic on dead human hair and other keratinized substrata in muck soil and freshwater, New York City, N. Y.

This species was first collected on January 30, 1947, when the temperature was very low. In fact, the muck soil was frozen solid at that time and had to be chopped from the ground. After thawing out in a culture dish in the laboratory, the soil was covered with filtered, sterile soil extract and baited with short segments of blond hair. The latter became infected within four days, which indicates that the chytrid develops very rapidly or was already present in the frozen soil in a reproductive stage. It was isolated later in May of the same year in freshwater from the same locality and grown extensively on dead human skin and other keratinized substrata. All attempts to grow it on cellophane, bleached grass leaf segments, chitin and several agar media have failed, which suggests that it may be specialized in its nutritional requirements.

Fundamentally *R. nodulosum* develops in the same manner as other well-known members of *Rhizophyidium*, so that it is unnecessary to give a detailed account and illustration of this process. Only the most outstanding stages and structural characteristics will be emphasized to distinguish this from other species of the genus. As shown in figure 1 bits of floating hair may become very thickly infected and almost covered with sporangia in various stages of development. In some instances infection was almost as great as reported previously (Karling, 5) for *Rhizophyidium keratinophilum* on the same substratum. Because of the thickness and refractive index of the hair, the endobiotic rhizoids are usually invisible, but when the chytrid is grown on thin, relatively transparent skin they are distinctly visible. They may be fairly short and fine or relatively coarse and extensive (FIG. 2), but in all thalli observed so far they were not very richly branched.

As noted in the diagnosis above, the outstanding structural characteristic is the irregular, nodular and gibbose shape of the



FIGS. 1-10. *Rhizophyidium nodulosum*.

sporangia, which in most cases is associated with the development of numerous prominent exit papillae. In the gibbose character of the sporangia this species resembles *R. gibbosum* (Zopf, 8; Karling, 4) to some degree, but in the latter species, which parasitizes various algae and rotifer eggs, only one exit papilla is formed. In

the young stages of *R. nodulosum* the sporangia are usually oval, slightly elongate to spherical in shape and contain a large central vacuole (FIG. 1A, 1B), but later a number of smaller additional vacuoles appear (FIG. 1C) which give the sporangium of this species a very characteristic internal appearance on hair. At this stage comparatively few conspicuous refractive globules are present in the cytoplasm (FIG. 1C), and the sporangia do not have the typical refringent appearance of most chytrids. The vacuolate condition persists for a long period and appears to be one of the most striking developmental phases. The multivacuolate stage is usually followed by one in which only a single large vacuole is present (FIG. 1D). At the same time the refractive material becomes more highly dispersed so that the protoplasm becomes greyish-granular in appearance (FIG. 1D, 1E, 1F). This appearance also is a striking, visible developmental stage of *R. nodulosum*. It is followed eventually by the coalescence of the refractive material to form the definitive globules of the zoospores (FIG. 1G, 1H, 2), and by cleavage (FIG. 1I, 1G).

In the meantime one to several prominent exit papillae develop which are usually very broad, fairly high, and occasionally elongate like a tube. During deliquescence, the outer wall may sometimes swell to remarkable proportions, as shown in figure 1H. As the tips open the zoospores ooze out in elongate masses (FIG. 1K), become active after a few seconds, and then dart away. Several exit papillae may deliquesce simultaneously so that several streams of zoospores may be emerging from the sporangium (FIG. 1L) at the same time. Zoospores which fail to emerge may often germinate *in situ* and develop into sporangia, as shown in figure 1O.

Another common occurrence in *R. nodulosum* is the development of small or large, irregular, simple or branched plugs or ingrowths from the wall of the sporangia. These become particularly conspicuous when the sporangia are empty (FIG. 1M, 9). These ingrowths, however, cannot be regarded as specific because similar ones have been described in species of *Chytriumyces* and other chytrids (Haskins, 2; Karling, 6).

So far resting spores have not been observed so that our knowledge of *R. nodulosum* is incomplete.

PARASITE OF RHIZOPHYDIUM NODULOSUM

During the course of this study of *R. nodulosum* its sporangia became infected by another minute chytrid which may prove to be an obligate parasite. At least it did not attack *Chytriomyces hyalinus*, *C. stellatus*, *Phlyctorhiza variabilis*, *R. keratinophilum*, *Karlingia rosea*, *Pythium debaryanum*, or *Aphanomyces lactis* which were growing luxuriantly on other substrata in the same cultures at the time of infection. Furthermore, it did not parasitize several species of filamentous and unicellular algae which were also present. For a period of time the infection of *R. nodulosum* was so extensive and severe that most of the thalli were killed. In several cases observed from ten to fifteen parasites were present on one sporangium (FIG. 11). However, the epidemic subsided within three weeks, and thereafter only sporadic infections occurred.

The parasite is characterized by narrowly obpyriform or citri-form, oval or elongate, epibiotic operculate sporangia which may be sessile or slightly stalked, by minute finely-branched or unbranched rhizoids, and by minute zoospores. Its epibiotic operculate sporangia and endobiotic simple or branched rhizoids distinguish it either as a species of *Chytriomyces* or *Chytridium*, but inasmuch as its resting spores are unknown its generic position is uncertain. The majority of *Chytridium* species are apophysate and form intramatrix resting spores in the position of the apophysis, whereas species of *Chytriomyces* may be apophysate or non-apophysate and form extramatrix resting spores. Since the parasite of *R. nodulosum* is non-apophysate and has a minute absorbing system it does not seem likely that its resting spores will be formed endobiotically as in most *Chytridium* species. On the other hand, its zoospores do not swarm in a vesicle after emerging from the sporangium as in species of *Chytriomyces*.

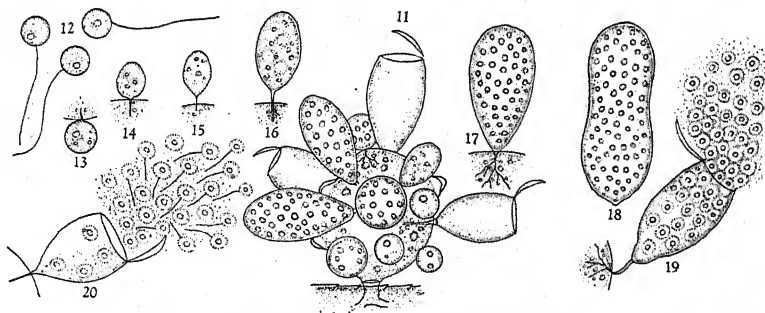
In the shape of the sporangia and the character of the endobiotic absorbing system it resembles somewhat *Chytridium versatile* (Scherffel, 7), *C. versatile* var. *acaulis*, *C. cocconeidis* (Canter, 1), and other questionable *Chytridium* species which parasitize algae, are non-apophysate, and have minute, branched or unbranched rhizoids. Resting spores are unknown in these *Chytridium* spe-

cies, and here also it is not likely that such spores will be found to occur endobiotically. Like the parasite of *R. nodulosum*, they are questionable members of *Chytridium* and, doubtless, will have to be removed from this genus when it is fully known. Although the parasite of *R. nodulosum* is somewhat similar to the species mentioned above, it differs in host range as well as by several minor structural characteristics, and for this reason it is regarded as a new species. It is included provisionally in the genus *Chytridium* and named *C. Rhizophydii* after the host which it parasitizes.

***Chytridium Rhizophydii* sp. nov.**

Fungus parasiticus. Sporangiis hyalinis, laevibus, citriformibus, ovalibus, $8-15 \times 12-22 \mu$ diam., aut obpyriformibus, $10-18 \times 22-32 \mu$ diam.; operculo hypocrateriformibus, $8-15 \mu$ diam. Zoosporis sphaericis, $2.2-2.8 \mu$ diam., globulo refractivo hyalino; flagello $12-14 \mu$ longo. Hyphis rhizomorphis eramosis, tenuissimis, $4-10 \mu$ longis. Sporidis perdurantibus ignotis.

Sporangia hyaline, smooth, elongately citriform or obpyriform, $10-18 \mu \times 22-32 \mu$, oval, $8-16 \times 12-22 \mu$, or elongate; operculum persistent, shallow saucer-shaped, $8-15 \mu$ diam. Zoospores spherical, $2-2.8 \mu$, with a minute hyaline refractive globule; flagellum $12-14 \mu$ long. Rhizoids unbranched, tapering and needle-like or finely branched, $4-10 \mu$ long. Resting spores unknown.



FIGS. 11-20. *Chytridium Rhizophydii*.

Parasitic on *Rhizophydium nodulosum*, New York, N. Y.

There is nothing unusual in the development of this parasite which distinguishes it sharply from other similar species of *Chytridium*, except that when the zoospores germinate at some distance

from the host the resulting sporangia become stalked as shown in figures 15, 16 and 19. The germinating spore or incipient sporangium is at first spherical (FIG. 13), but it soon elongates and becomes narrowly oval or broadly fusiform (FIG. 15, 16). The mature sporangia are usually of this shape with tapering ends, but in numerous cases they may become narrowly obpyriform (FIG. 17, 19) or even broadly fusiform. The apical operculum is unusually large, and may be only slightly less in diameter than the sporangium (FIG. 20). Particularly noteworthy is the dehiscence of the sporangium. This may often occur with such force that it resembles an explosion. As a large mass of spores is shot out, the operculum is pushed up and back with such speed that its movement is usually invisible. In some cases observed the discharge of the zoospores was so forceful that the sporangium recoiled and broke loose from the attached rhizoid. Figure 19 shows a sporangium in the process of dehiscence with the stalk breaking loose at the surface of the host cell. However, the force required to break the rhizoid is not very great because sporangia may frequently be torn loose in mounting the material for microscopic study (FIG. 18). If such sporangia are mature they will dehisce and discharge zoospores in the usual manner. The discharged zoospores lie quiescent in a slimy matrix for a few seconds and then free themselves and dart away.

As noted before resting spores have not been found in this parasite. Consequently, its life cycle is not fully known, and it is not certain whether it is a species of *Chytridium* or *Chytriomycetes*.

SUMMARY

Rhizophydium nodulosum sp. nov. is a keratinophilic saprophyte which was isolated from frozen muck soil and freshwater from Van Cortlandt Park, New York City. It grows readily on keratinized substrata such as hair and skin but not on chitin, cellulose, or various types of nutrient agar media. It is characterized structurally by hyaline, smooth, nodular and angular zoosporangia with several prominent exit papillae which give the sporangia their typical shape and appearance. Resting spores are unknown.

Its sporangia may become heavily parasitized by another small chytrid which is characterized by elongately citriform, obpyriform,

oval or elongate, operculate sporangia and minute branched or unbranched rhizoids. The resting spores of the parasite are unknown so that it is not certain whether this chytrid is a species of *Chytridium* or *Chytriomycetes*. Nevertheless, it is included provisionally in *Chytridium* and named *Chytridium Rhizophydii*.

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DESCRIPTION OF FIGURES

FIGS. 1-10. *Rhizophyidium nodulosum*. FIG. 1. Segment of human hair infected with 23 thalli in various stages of development. $\times 860$. FIG. 2. Complete thallus from dead human skin. $\times 900$. FIG. 3. Zoospores in active swimming stage. $\times 1,720$. FIG. 4. Amoeboid zoospores. $\times 1,720$. FIGS. 5-8. Zoospores at rest and successive germination stages. $\times 1,720$. FIG. 9. Empty sporangium with secondary sporangia developing *in situ*. $\times 1,000$. FIG. 10. Large irregular empty sporangium with five ingrowths or plugs of material from the wall. $\times 1,000$.

FIGS. 11-20. *Chytridium Rhizophydii*. FIG. 11. Sporangium of *R. nodulosum* parasitized by twelve thalli. $\times 1,800$. FIG. 12. Zoospores in active swimming stage. $\times 2,500$. FIGS. 13-16. Germination stages and development of thalli with unbranched, needle-like rhizoids. $\times 2,800$. FIG. 17. Narrowly obpyriform sporangium with branched rhizoids. $\times 1,600$. FIG. 18. Detached elongate, slightly constricted sporangium. $\times 1,600$. FIG. 19. Discharge of zoospores. $\times 1,600$. FIG. 20. Dissemination of zoospores. $\times 1,600$.

A NEW SPECIES OF ACHLYA

ARTHUR WILLIAM ZIEGLER¹

(WITH 10 FIGURES)

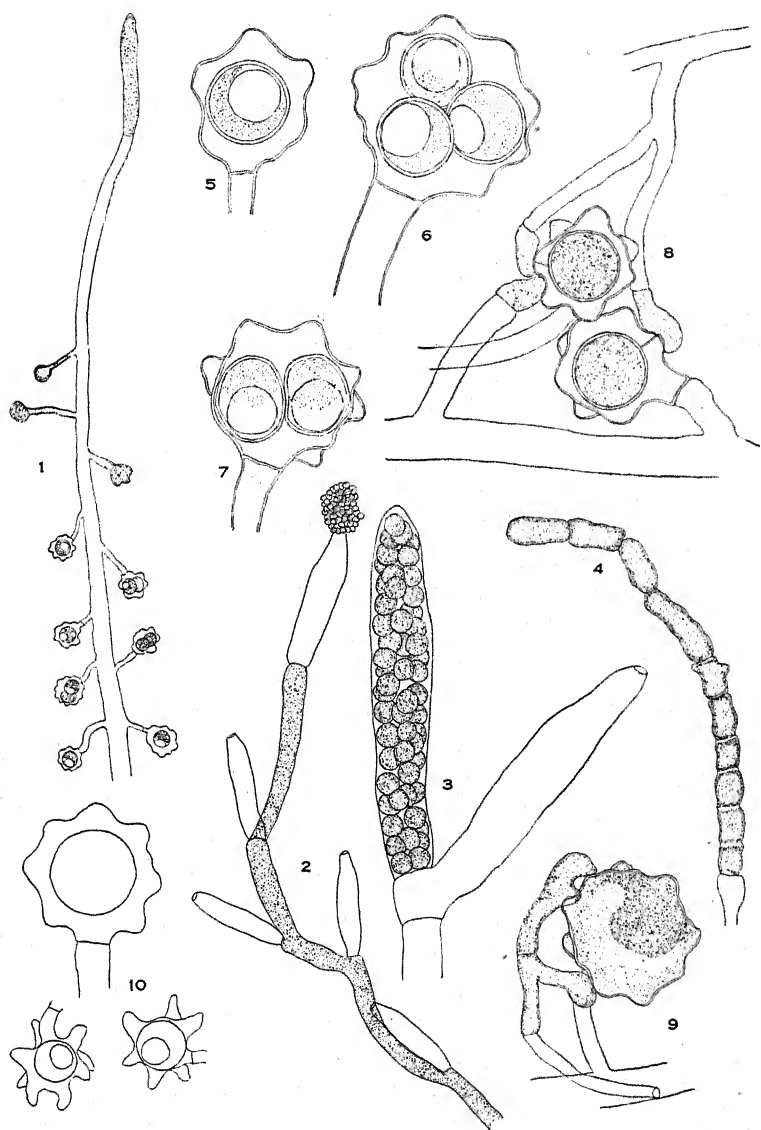
A new species of *Achlya* was found in a water collection made from a ditch near the pump at Kalmia Gardens, Hartsville, South Carolina.

Achlya crenulata n. sp.

Mycelium modice densum, hyphae usque ad 1 cm. longae in seminibus *Cannabis sativae*, diam. culturae plerumque 2-2.5 cm. Sporangia copiosa, longa, cylindrica, in medium ferme 271.4μ longa et 37.6μ lata. Ejectio sporae typica generis. Gemmae copiosae, efformatae hypha transformata in seriem cellularum brevium cylindricarum. Oogonia globosa, ex 33.3μ ad 60μ in diam., plerumque ferme 48.6μ , obtusis papillis tecta. Paries terminalis papillarum vel tenuior vel eadem crassitudine. Longitudo caulis oogonii ferme aequalis latitudini oogonii. Ova eccentrica, 1-3, rare 5, $22.2-30.7\mu$, plerumque 27.6μ in diam., saepe ellipsoidea, non complentes oogonium. Antheridia rara, plerumque origine diclina sed interdum androgyna, reperta in ferme uno ex 14 oogoniis, omnino vacua antequam ova matura sunt.

Mycelial growth moderately dense, hyphae reaching a length of 1 centimeter on hemp seed, diameter of culture about 2 to 2.5 cm. Sporangia plentifully formed, long, cylindrical, averaging 271.4μ by 37.6μ . Spore discharge typical for the genus. Gemmae plentiful in cultures, formed by the transformation of a hypha into a row of short cylindrical cells. Oogonia spherical, ranging from $33.3-60\mu$, mostly about 48.6μ , covered with blunt crenulate projections. The end wall of the projections may or may not be thinned. Length of oogonial stalk about equal to the width of the oogonium. Eggs eccentric, 1-3, occasionally 5, $22.2-30.7\mu$, mostly 27.6μ in diameter, frequently ellipsoid in shape, not filling the oogonium. Antheridia rarely formed, usually diclinous but occasionally androgynous, occurring on about 1 out of every 14 oogonia, and becoming completely empty before the eggs are mature. Antheridial tube usually present.

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FIGS. 1-10. *Achlya crenulata*.

In water on hemp seed bait. Water collected by the author in a ditch near the pump at Kalmia Gardens, Hartsville, South Carolina, February 9, 1947.

This species was thought to be *A. glomerata* when the author first examined it but further study showed that the oogonia were much larger and were not in clustered groups as in *A. glomerata* (1). Figure 10 shows the relative sizes of these two species.

Achlya crenulata differs from *A. radiosa* Maurizio (1) in its blunt papillae and smaller egg-size and from *A. recurva* Cornu (4) in the general shape of the spines, number of eggs, and few antheridia. It can be distinguished from *A. abortiva* Coker and Braxton (2) in its round and not oblong oogonia, shorter stalk, and by all the eggs reaching maturity.

This new species germinates readily after a rest period of six weeks if it is placed in fresh water. A short germ tube is formed with an apical sporangium which discharges its spores in the typical *Achlya* manner.

As previously stated, antheridia occur on about one out of every fourteen oogonia. In order to induce, if possible, a greater antheridial growth, the fungus was grown in several different solutions which favored antheridial branches as used by Kauffman (3). The results were as follows:

1. Mushroom grub.

Mycelium scanty after seven days growth. Long rows of typical gemmae formed. Very few oogonia formed, all typical, no antheridia present. Oogonial and egg measurements as above.

2. 0.05% haemoglobin and 0.2% potassium phosphate.

Growth heavy after seven days, culture 40 mm. in diameter on hemp seed, sporangia abundant, typical gemmae formed abundantly. No sex organs. Twenty-four hours after pure water added, gemmae had germinated to form sporangia. After seventy-two hours abundant oogonia present, antheridia on every third oogonium. Antheridial branches not profusely branched. No change after 120 hours. Oogonial and egg measurements as above.

3. 0.05% haemoglobin and 0.2% potassium nitrate.

After seven days, growth sparse, culture 15 mm. in diameter on hemp seed, few sporangia formed, typical gemmae moderately abundant. No sex organs formed. Twenty-four hours after pure water added gemmae had germinated to form sporangia. After seventy-two hours sex organs formed abundantly. Diclinous antheridia on about every seventh oogonium. Eggs mostly mature. After 120 hours eggs mature—no other change. Oogonial and egg measurements typical for species.

4. 0.05% haemoglobin and 0.1% potassium phosphate with 0.1% potassium sulphate.

After seven days growth was sparse but diameter of colony on hemp seed was 30 mm. Sporangia few, gemmae moderately abundant. No sex organs formed. Twenty-four hours after placing in fresh water one fourth of gemmae had germinated to form sporangia. After seventy-two hours sex organs plentifully formed. Diclinous antheridial branches on every fifth oogonium. After 120 hours no change except eggs mature. Measurements typical for species.

5. 0.05% haemoglobin with 0.1% potassium phosphate and 0.1% sodium chloride.

After seven days growth was heavy. Culture 22 mm. in diameter on hemp seed. Few sporangia formed, gemmae very abundant and could be seen by the naked eye. No sex organs formed. Twenty-four hours after placing in fresh water gemmae germinated to form sporangia. Young oogonia formed with about three-fourths with diclinous, much branched antheridia. After seventy-two hours oogonia abundant and eggs mostly mature. Antheridia with long profuse branches applied to every third oogonium. No change after 120 hours. No change in measurements.

6. 0.05% haemoglobin with 0.1% disodium hydrogen phosphate and 0.1% potassium sulphate.

Growth after seven days moderately abundant. Culture on hemp seed 35 mm. in diameter. Sporangia few, gemmae moder-

ately abundant. No sex organs formed. After twenty-four hours in fresh water one half of the gemmae had germinated to form sporangia. After seventy-two hours no change. After 120 hours a few young oogonia were formed but no antheridia present. Measurements typical.

7. 0.05% leucin.

After seven days growth was moderately abundant. Diameter of culture on hemp seed 30 mm. No sporangia formed and few gemmae. No sex organs. Twenty-four hours after placing in fresh water gemmae formed sporangia. After 72 hours a few young oogonia formed. Antheridial branches profuse but only applied to every other oogonium. After 120 hours eggs mature, antheridial branches formed profusely although not as profusely as in number nine. Egg and oogonium measurements typical.

8. 0.05% leucin plus 0.1% potassium phosphate.

Growth after seven days heavy. Diameter of culture on hemp seed 25 mm. Sporangia very abundant. Gemmae abundant. No sex organs. After twenty-four hours in fresh water all gemmae had germinated to form sporangia. After seventy-two hours young oogonia formed abundantly. Antheridia found on about every twelfth oogonium. After 120 hours eggs mature—no further change. Measurements typical.

9. 0.05% leucin plus 0.1% calcium phosphate.

Growth after seven days sparse, hyphae translucent. Culture on hemp seed 10 mm. in diameter. No sporangia or gemmae formed. No sex organs. Width of hyphae small. Twenty-four hours after placing in fresh water, no change. After seventy-two hours no change. After 120 hours oogonia with eggs moderately abundant with best production of diclinous antheridia seen in any solution. Antheridia on all oogonia. Antheridial branches long, twining and branched. Measurements typical.

SUMMARY

A new species of *Achlya*, *A. crenulata*, is described. It differs primarily from other eccentric species of *Achlya* in that its spheri-

cal oogonia are covered with blunt crenulate projections. The fungus was grown in several media: 0.05% haemoglobin with 0.2% potassium phosphate gave the best vegetative growth. A solution of 0.05% leucin with 0.1% calcium phosphate restricted the vegetative growth but after 120 hours in fresh water antheridia were more profusely formed than in any other solution.

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EXPLANATION OF FIGURES

FIG. 1. Habit of growth, $\times 178$. FIG. 2. Arrangement of sporangia, $\times 178$. FIG. 3. Sporangium about to discharge its spores, $\times 1,128$. FIG. 4. Typical group of gemmae, $\times 178$. FIGS. 5, 6, 7. Oogonia with eggs, $\times 1,128$. FIG. 8. Oogonia showing declinuous antheridia and antheridial tubes, $\times 1,128$. FIG. 9. Immature oogonium showing egg formation and declinuous antheridia, $\times 1,128$. FIG. 10. Outline drawings of *A. crenulata* (above), and *A. glomerata* (below).

HELMINTHOSPORIUM PORTULACAE A NEW PATHOGEN OF PORTULACA OLERACEA L.

WM. E. RADER

(WITH 1 FIGURE)

Portulaca oleracea L. or purslane is a well known weed distributed throughout the temperate United States. It occurs in great abundance in the muckland areas of New York State which are devoted largely to the production of vegetable crops. Purslane is now controlled by pulling and removing the plants from the fields by hand. Because of the rapidity with which this weed re-establishes itself from pieces of stems and the large numbers of seeds produced, its eradication involves considerable time and expense each year.

During 1944 investigations were conducted to determine the possibility of controlling this weed in the field by means of artificially disseminating the fungus *Dichotomophthora portulacae* Mehrlich and Fitzpatrick.¹ Near the test plots at Watkins Glen, New York, another fungus was found attacking the purslane. During 1945 this same fungus was found killing purslane in widely separated areas in New York State. Isolations from the diseased plants have consistently yielded a fungus which is believed to be new.

Greenhouse and field tests with *Dichotomophthora portulacae* and this new pathogen indicate that under the dry conditions which prevail in New York State during the summer these fungi will be of little value in controlling this weed. However, because of the possibility of using either of these fungi for the biological control of *Portulaca oleracea* in regions of high rainfall and humidity, this new disease together with its pathogen are described.

¹ Mehrlich, F. P., and H. M. Fitzpatrick. *Dichotomophthora portulacae* a pathogene of *Portulaca oleracea*. *Mycologia* 27 (5): 543-550. 1935.

DESCRIPTION OF THE DISEASE

Symptoms appear in two to five days on plants artificially inoculated with spore suspensions. The brownish, watersoaked lesions which appear along the stem may involve the whole branch or only isolated areas. The smaller lesions spread rapidly and soon cover all of the stem. The diseased areas change to a dark brown color and finally become black. Conidia of the fungus are produced in large numbers on the blackened areas on the stems and leaves.

MORPHOLOGY OF THE PATHOGEN

The brown septate hyphae of the pathogen are 6 to 7.5 μ in diameter, and ramify intercellularly throughout all of the susceptible tissues.

TABLE 1
PERCENTAGE DISTRIBUTION OF CONIDIAL TYPES OF *Helminthosporium portulacae* OCCURRING ON *Portulaca oleracea* AND POTATO DEXTROSE AGAR¹

Spore Class	<i>P. oleracea</i>	Potato Dextrose Agar
3-celled	2.35	2.63
4-celled	1.18	5.26
5-celled	2.35	2.63
6-celled	3.53	11.84
7-celled	11.76	15.79
8-celled	9.41	19.74
9-celled	18.82	23.68
10-celled	29.41	9.30
11-celled	9.41	7.89
12-celled	4.71	—
13-celled	5.88	—
14-celled	1.18	—

¹ Based on measurements of 250 spores.

In culture on potato dextrose agar the mycelium is at first hyaline, sparse and appressed to the medium. The colony soon becomes olivaceous brown and then black, profusely dotted with small bulbils. The hyphae in culture are 5–7 μ in diameter, septate and anastomose sparingly.

The conidia are brown, straight or slightly curved, elongate, rounded at the ends, 3–14 (mostly 9–10) celled (Table 1) and average $110.2 \times 12.8 \mu$ (Table 2).

The conidia are borne in fascicles of three to ten on brown, septate conidiophores 40–200 μ long. The conidiophores are sim-

TABLE 2
SIZE OF CONIDIA IN MICRONS OF *Helminthosporium portulacae*¹

Spore Class	Length	Width
3-celled	36.6	10.0
4-celled	43.3	10.4
5-celled	65.6	11.9
6-celled	82.1	11.7
7-celled	94.9	12.5
8-celled	112.9	13.8
9-celled	115.9	13.7
10-celled	141.3	13.5
11-celled	141.2	13.2
12-celled	171.0	13.1
13-celled	173.5	14.1
14-celled	183.2	14.7

¹ Based on a mean of at least 15 spores in each class.

ple or occasionally branched and are produced singly or in clusters on the stems of *Portulaca* sp. or on culture media.

The bulbils are black, globose or irregular in shape and 53–276 μ (ave. 114.3 μ) in diameter, black without, hyaline to gray within.

No perfect stage of the fungus has been found. The characters detailed above appear to place this organism in the genus *Helminthosporium*. A search of the literature reveals no species of the genus parasitic on any of the species of *Portulacaceae*. Neither do the characters of the pathogen approach closely any of the described species of *Helminthosporium*. A new species is therefore established, the formal description as follows:

***Helminthosporium portulacae* sp. nov.**

Mycelium septatum, primum hyalinum, actutum olivaceo fuscum vel nigro fuscum evadans. Conidiophoria fusca, simplicia, in substrato singulatim aut gregatim portata, 40–200 μ longitudine \times 5–7 μ diametro. Cellae terminales conidiophorium levidensis contortae, ferentes 3–10 conidia fusca, elongata, recta vel levidensis curvata, in terminis orbiculata, cellis 3–14, plerumque 9 aut 10, 29.3–183.3 \times 9.1–16.5 μ (medio 110.2 \times 12.8 μ). Bulbiles abundantes, minuti, extra nigri, intus hyalini vel cani, forma sphaerici vel irregulares, 53–276 μ (medio 114.3 μ) diametro.

Domicilium: biogenum in *Portulaca oleracea* L.

Mycelium septate, at first hyaline, soon becoming olivaceous brown to dark brown. Conidiophores brown, simple, borne singly or in groups upon the substratum, 40–200 $\mu \times$ 5–7 μ in diameter. Terminal cells of the conidiophores slightly contorted, bearing 3–10 brown, elongate, straight or slightly curved conidia, which are rounded at the ends, 3–15 (mostly 9–10) celled, 29.3–183.3 \times

9.1–16.5 μ (ave. 110.2 \times 12.8 μ). Bulbils abundant, minute, black without, hyaline to gray within, spherical to irregular in shape, 53–276 μ (ave. 114.3 μ) in diameter.

Habitat: biogenous on *Portulaca oleracea* L.

Type locality: Watkins Glen, New York.

Type material has been deposited in the herbarium of the De-

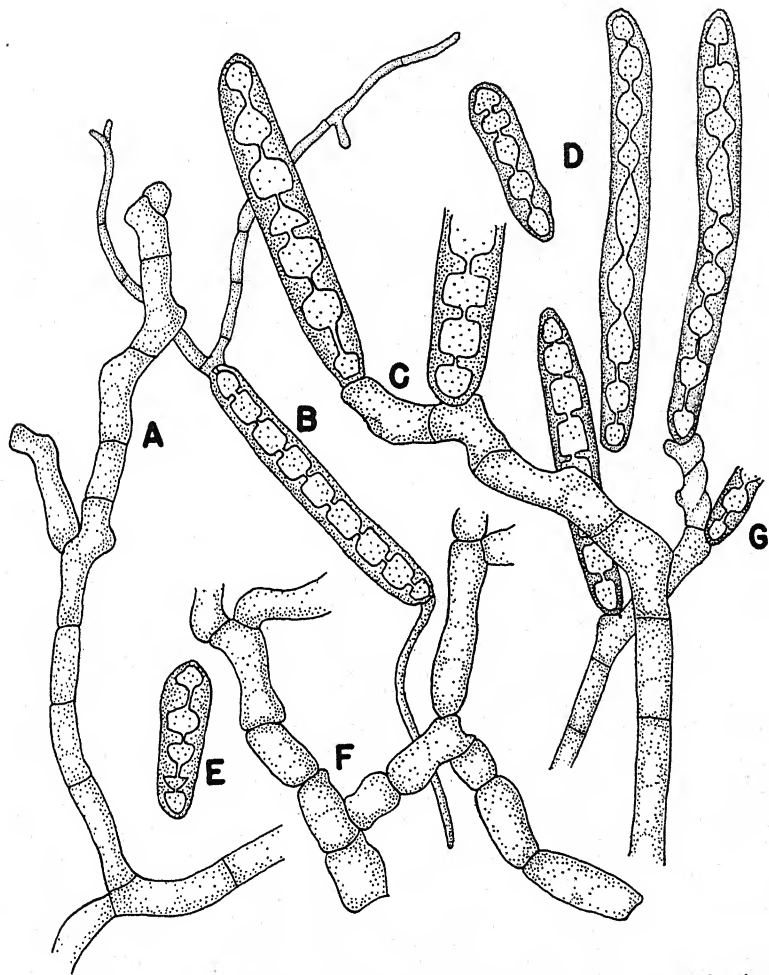


FIG. 1. *Helminthosporium portulacae*, a, conidiophore; b, germinating conidium; c and g, conidiophores showing mode of attachment of conidia; d and e, five- and nine-celled conidia; f, anastomosing hyphae, all drawn with the aid of a camera lucida, \times 450.

partment of Plant Pathology, Cornell University, Ithaca, New York. Cultures have been sent to the American Type Culture Collection, Washington, D. C., and to the Centraalbureau voor Schimmelcultures, Baarn, Nederland.

ACKNOWLEDGMENT

The writer is indebted to Mrs. M. W. Allen of the Botany Department, Cornell University, for assistance in the preparation of the Latin diagnosis.

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HETEROTHALLIC ANTIBIOSIS IN MUCOR RACEMOSUS¹

HUBERT A. HARRIS

During a preliminary testing of stock laboratory cultures of molds for possible antibiotic properties, a culture of a phycomycete, *Mucor racemosus* Fres., indicated antibacterial effects. It was not known whether this culture represented a plus or a minus strain of the heterothallic fungus. Inasmuch as antibiosis has been reported to occur very infrequently in the phycomycetes, the antibiotic action of *M. racemosus* was investigated further.

Wilkins and Harris (3) tested 19 phycomycetes for antibiotic action using *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas pyocyanea* as test organisms. Negative results were obtained with all of these phycomycetes except for *Phytophthora erythroseptica* which exhibited antibacterial action against all three test organisms. Antibiotic tests also included heterothallic strains of nine different species of Mucoraceae. Among these were plus and minus strains of *Mucor hiemalis*, *M. mucedo*, and *M. sexualis*.

Waksman and Horning (2) tested six cultures of known species of *Rhizopus* against *Bacillus subtilis* with negative results.

METHODS AND MATERIALS

The strains of the fungus and the bacteria used in this investigation are as follows:

- M. racemosus*: plus strain, ATCC 1216a
- M. racemosus*: minus strain, ATCC 1216b
- E. coli*: ATCC 9739
- S. aureus*: Oxford strain H, ATCC 9144
- S. lutea*: stock laboratory culture, source unknown
- S. marcescens*: ATCC 4261.

¹ Presented in abstract form at the Meeting of the Colorado-Wyoming Academy of Science, Colorado Springs, May, 1947.

To determine possible antibiotic differences between the plus and minus strains of *M. racemosus*, the cylinder plate method of Abraham *et al.* (1), which is used with modification by many investigators of antibiotics, afforded a means for obtaining comparative data.

Plus and minus strains of the fungus were cultivated in 125 ml. Erlenmeyer flasks containing 50 ml. of Sabouraud's broth autoclaved at 15 pounds pressure for 20 minutes. The flasks were inoculated with a mycelial transfer of the fungus grown on a Sabouraud agar plate for three days at room temperature (23–27° C.). The inoculated flasks were incubated at room temperature for 10–12 days.

The contents of the flasks were thoroughly shaken and the culture liquid filtered twice through No. 1 Whatman filter paper prior to filtration through a medium (N) Berkefeld filter. Difco Bacto-Yeast Beef Agar, autoclaved at 15 pounds pressure for 20 minutes, was dispensed (25 ml.) into sterile petri dishes with a sterile pipette. The surface of the hardened agar was flooded with a 16–20 hour culture suspension of the test bacterial organism and the excess suspension was pipetted off. The bacterial test organisms were transferred three times on successive days to 10 ml. of nutrient broth and incubated at 37° C. save *S. marcescens* which was incubated at room temperature.

The surfaces of the flooded agar plates, with the covers removed, were dried for one hour in the 37° C. incubator. Four stainless steel penicillin assay cylinders (0.8 mm. O. D., 0.6 mm. I. D., and 10.0 mm. in length) were warmed in a flame and spaced equidistantly around the circumference of the plate. Alternate cylinders were then filled, respectively, with the plus and minus strain filtrates. Five plates, containing duplicate tests of the filtrates, were made for each of the bacterial test organisms. The test plates were incubated at the same temperatures employed for cultivating the various bacteria in nutrient broth.

Measurements were made to the nearest 0.5 mm. of the outside diameters of the inhibitory zones of the test organisms by means of pointed dividers and under a magnifying lens.

To test the thermostable property of the minus strain filtrate,

the paper filtrate was autoclaved at 15 pounds pressure for 30 minutes and then cooled prior to filling the cylinders.

EXPERIMENTAL RESULTS

Antibiotic tests: The results of the effects of the plus and minus strain filtrates of *M. racemosus* on the two Gram positive bacteria, *S. lutea* and *S. aureus*, and on the two Gram negative bacteria, *E. coli* and *S. marcescens*, are shown in Table 1.

TABLE 1

ANTIBACTERIAL EFFECTS OF PLUS AND MINUS STRAINS OF *M. racemosus*
(MEASURED IN MM. OF INHIBITION ZONES OF DUPLICATE TESTS)

Test Organism	Plate Number	Culture Filtrate				Minus Strain Average
		Minus Strain		Plus Strain		
<i>S. marcescens</i>	1	13.5	13.5	0	0	13.6
	2	14.0	14.0	0	0	
	3	13.0	13.5	0	0	
	4	14.0	13.5	0	0	
	5	13.5	13.5	0	0	
<i>S. lutea</i>	1	23.0	19.0	0	0	18.5
	2	15.0	17.5	0	0	
	3	20.5	17.5	0	0	
	4	17.0	a	0	0	
	5	19.5	18.0	0	0	
<i>E. coli</i>	Negative results					
<i>S. aureus</i>	Negative results					

a—no measurement due to cylinder leak.

These data show that the antibiotic activity of *M. racemosus* is restricted to the minus strain of the fungus for the organisms tested. The antibiotic action of the minus strain filtrate was somewhat greater for *S. lutea* than for *S. marcescens*, and no antibiotic effect resulted with either *E. coli* or *S. aureus*.

Thermostability tests: Determinations of the thermostability of the antibiotic principle in the minus strain filtrate by autoclaving and the results obtained with *S. marcescens* and *S. lutea* are presented in Table 2.

TABLE 2
EFFECT OF AUTOCLAVING ON THE ANTIBACTERIAL PROPERTIES OF THE MINUS STRAIN FILTRATE OF *M. racemosus* (MEASURED IN MM. OF INHIBITION ZONES OF DUPLICATE TESTS)

Test Organism	Plate Number	Culture Filtrate (Minus Strain)			
		Non-autoclaved		Autoclaved	
<i>S. marcescens</i>	1	11.0	11.5	11.0	12.0
	2	11.5	12.0	11.0	10.5
	3	11.5	11.5	10.5	10.5
	4	11.0	11.5	11.0	10.5
	5	10.0	10.0	11.0	11.5
	Average	11.1		10.9	
<i>S. lutea</i>	1	19.0	18.0	17.0	17.5
	2	17.5	18.5	18.0	18.5
	3	19.0	18.0	20.0	19.5
	4	16.5	17.0	17.5	19.0
	5	19.0	18.5	19.5	19.0
	Average	18.1		18.5	

These results show that the antibiotic principle of the minus strain filtrate is thermostable and no significant differences occurred with autoclaved or non-autoclaved filtrates. Again, a greater antibiotic effect is evident for *S. lutea* than for *S. marcescens*.

SUMMARY

The phycomycete, *Mucor racemosus*, possesses antibiotic properties for certain bacteria. The active antibiotic principle is

thermostable and is restricted to the minus strain of the heterothallic fungus for the bacteria tested.

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NOTES ON SOME CERCOSPORAE OF INDIA

M. J. THIRUMALACHAR AND CHARLES CHUPP

Species of *Cercospora* included in the present study were collected in Mysore State, South India, during 1944-46. Most of the collections were made in the vicinity of Bangalore and Nandi Hills which are situated at an altitude of 3,000 ft. and 4,850 ft. above sea level respectively. The annual rain fall is about thirty inches. The present collection comprises thirty-nine different species of which twelve are described as new. Many of the commonly known species of *Cercospora* are not included. Types of the new species are deposited in the Herb. Crypt. Ind. Orient., New Delhi, India, Cornell University Herbarium, and in Herb. I. M. I., Kew, England.

Cercospora Achyranthina sp. nov.

Maculae circulares, 0.5-2 mm. diam., centro alutaceo usque griseo, margine rubro usque badio; fructificatio amphigena, praecipue epiphylla; stromata fusca e cellulis paucis usque $25\ \mu$ in diam.; caespituli ex hyphis 2-10 divergentibus compositi; conidiophora pallide olivaceo-brunnea, apices versus pallescentia attenuataque, sparse septata, non ramosa, recta vel flexuosa, 0-5 geniculata, apice anguste subtruncata, $4-5.5 \times 15-80\ \mu$, plerumque $25-45\ \mu$; conidia hyalina, acicularia, recta vel subcurvata, indistincte multiseptata, basi truncata, ad apicem acuta, $2.5-5 \times 35-130\ \mu$.

Leaf spots circular, 0.5-2 mm. in diam., center tan to gray, margin red to reddish brown; fruiting amphigenous but chiefly epiphyllous; stromata dark brown, a few cells to $25\ \mu$ in diam.; fascicles 2-10 divergent stalks; conidiophores pale olivaceous brown, paler and narrower toward the tip, sparingly septate, not branched, straight to bent, 0-5 geniculate, narrowly subtruncate tip, $4-5.5 \times 15-80\ \mu$, mostly $25-45\ \mu$; conidia hyaline, acicular, straight to slightly curved, indistinctly multiseptate, base truncate, tip acute, $2.5-5 \times 35-130\ \mu$.

On leaves of *Achyranthis aspera* L., Bangalore, 20-8-1944, leg. M. J. Thirumalachar. The species is distinct from *C. Achyranthis* and *C. centrostachydis* known on the same host genus.

CERCOSPORA APII Fres. Beiträge zur Mycol. Drittes Heft, p. 9. 1863.

C. penicillata var. *Apii* Fekl., Hedwigia 2: 132-136. 1863.

On leaves of *Apium graveolens* L., Bangalore, 12-12-1945, leg. M. J. Thirumalachar.

CERCOSPORA ASPLENI Jaap. Ann. Mycol. 14: 43, 1916.

On leaves of *Asplenium nidus*, Bangalore, 29-7-1944, leg. M. J. Thirumalachar. The collections of the fungus were made in the botanical garden of Central College, Bangalore. The large fronds were severely blemished in some cases.

Cercospora bangalorensis sp. nov.

Maculae obscurae vel atrae, subcirculares vel irregulares, 0.5-3 mm. in diam., interdum margine dilute flavidulo cinctae; fructificatio amphigena; stromata carentia vel cellulis paucis fuscis composita; caespituli ex hyphis 2-12 divergentibus consistentes; conidiophora pallide vel perpallide fuliginea, colore uniformia, non vel e basi apicem versus conspicue attenuata, dense septata saltem ad basin, non ramosa, recta vel solum leniter curvula vel flexuosa, rare geniculata, ad apicem conica, $3-5 \times 10-65 \mu$; conidia pallide vel perpallide olivacea, anguste obclavata, recta vel leniter curvata, 1-5-septata, basi longe obconica, apice obtuso vel subacuta, $3-4.5 \times 20-60 \mu$.

Leaf spots dark to black, subcircular to irregular, 0.5-3 mm. in diam., sometimes with faintly yellowish margin; fruiting amphigenous; stromata lacking or composed of a few dark brown cells; fascicles of 2-12 divergent stalks; conidiophores pale to very pale fuliginous, uniform in color, none to marked attenuation from base to tip, closely septate at least near the base, not branched, straight or only slightly curved or bent, rarely geniculate, tips conical, $3-5 \times 10-65 \mu$; conidia pale to very pale olivaceous, narrowly obclavate, straight to slightly curved, 1-5-septate, long obconical base, tips obtuse to subacute, $3-4.5 \times 20-60 \mu$.

On the leaves of *Aristolochia indica* L., Bangalore, 20-8-1944, leg. M. J. Thirumalachar.

CERCOSPORA CALOTROPIDIS Ell. & Ev. Missouri Bot. Gard. Ann. Rept. 9: 120. 1898.

On leaves of *Calotropis gigantea* Br., Bangalore, 13-9-1945, leg. M. J. Thirumalachar.

CERCOSPORA CANESCENS Ell. & Mart. Amer. Naturalist 16: 1003. 1882.

Cercosporina Kikuckii Mats. & Tomoy., Ann. Phytopath. Soc. Japan 1 (6): 1. 1925.

Cercospora Vignicaulis Tehon, Mycologia 29: 436. 1937.

On leaves of *Vigna catjang* Endl., Bangalore, 22-12-1944, leg. M. J. Thirumalachar.

CERCOSPORA CANNABIS Hara & Fukui

Cercosporina Cannabis Hara, Phytopathology of crop plants, p. 195. 1928.

On leaves of *Cannabis sativa* L., Goribidnur, Mysore, 20-12-1944, leg. Thirumalachar.

CERCOSPORA CAPPARIDIS Sacc. Nuovo Giorn. Bot. Ital. 8: 189. 1876.

On leaves of *Capparis horrida* L., Bangalore, 2-9-1945, leg. M. J. Thirumalachar.

CERCOSPORA CARBONACEA Miles. Trans. Ill. Acad. Sci. 10: 255. 1917.

On *Dioscorea alata* ?, Nandi Hills, 19-7-1944, leg. M. J. Thirumalachar.

***Cercospora Clauseniae* sp. nov.**

Maculae irregulares usque angulares, 0.5-4 mm. vel in areas magnas coalescentes, primum uniformiter atrobadae, demum centro pallide brunneo usque sordide griseo; fructificatio epiphylla; stromata subglobosa, brunnea, 20-50 μ in diam.; caespituli densi, divergentes; conidiophora pallide vel pallidissime olivaceo-brunnea, colore uniformia, latitudine irregularia, 1-5-septata, rare geniculata, non ramosa, apice obtuso, 4-5.5 \times 10-65 μ ; conidia hyalina usque dilute olivacea, cylindro-obclavata, interdum distincte cylindrica, recta usque leniter curvata, multiseptata, basi longe obconice truncata, apice subobtusio, 3-5.5 \times 30-165 μ .

Leaf spots irregular to angular, 0.5-4 mm. or coalescing into large areas, at first uniformly dark reddish-brown, later the center becoming pale brown to dingy gray; fruiting chiefly epiphyllous; stromata subglobular, brown, 20-50 μ in diam.; fascicles dense, divergent; conidiophores pale to very pale olivaceous brown, uniform in color, irregular in width, 1-5-septate, rarely geniculate, not branched, blunt apex, 4-5.5 \times 10-65 μ ; conidia hyaline to

faintly olivaceous, cylindro-obclavate, occasionally distinctly cylindrical; straight to slightly curved, multiseptate, base long obconically truncate, tip subobtuse, $3-5.5 \times 30-165 \mu$.

On the leaves of *Clauseria Willdenowii* Wight & Arn., Nandi Hills, Mysore, 18-12-1945, leg. M. J. Thirumalachar.

CERCOSPORA COCCULI Syd. Ann. Crypt. Exot. 2: 264. 1929.

On leaves of *Cocculus villosus* DC, Bangalore, 2-9-1945, leg. M. J. Thirumalachar.

CERCOSPORA COFFEICOLA Berk & Br. Grevillea 9: 99. 1881.

On leaves of *Coffea arabica* L., Bangalore, 5-12-1944, leg. M. J. Thirumalachar.

CERCOSPORA CONSIMILIS Syd. Ann. Mycol. 23: 423. 1945.

On leaves of *Vernonia* sp., Koppa Road, Mysore, 2-4-1945, leg. M. J. Thirumalachar. This species should probably be considered as an *Helminthosporium*.

CERCOSPORA FUSIMACULANS Atk. Jour. Elisha Mitchell Sci. Soc. 8: 50. 1892.

Cercospora Panici Davis, Wisc. Acad. Trans. 19: 714. 1919.

C. Panici-milacei Sawada, Descr. Catal. Formosan F. V. Rept. 51: 31. 1931.

On the leaves of *Panicum javanicum* Poir., Bangalore, 2-9-1945, leg. Thirumalachar.

CERCOSPORA HIBISCINA Ell. & Ev. Proc. Acad. Nat. Sci. Phila. 47: 438. 1895.

On leaves of *Hibiscus Cannabinus* L., Bangalore, 28-12-1944, leg. Thirumalachar.

***Cercospora Holarrhenae* sp. nov.**

Maculae atrobadiæ, usque fere nigra, angulares, nervis marginatae, 2-8 mm. in diam. vel in areas magnas coalescentia; fructificatio plerumque epiphylla; stromata fusca usque atra subglobosa, 20-60 μ in diam.; caespituli intense fuliginei, apicem rotundatum versus pallescentes et angustiores, rare septati, non geniculati, recti vel undulantes, $2-4 \times 10-40 \mu$, conidia subhyalina usque pallidissime olivacea, anguste obclavata vel interdum cylindrica, recta vel curvata, indistincte multiseptata, basi brevi obconice truncata, apice subacuto vel conico, $2-4 \times 20-75 \mu$.

Leaf spots dark reddish brown to almost black, angular, bounded by the leaf veins, 2–8 mm. in diam. or coalescing into large areas; fruiting chiefly epiphyllous; stromata dark brown to black, subglobular, 20–60 μ in diam.; fascicles very dense, slightly divergent; conidiophores pale to very pale fuliginous, paler and more narrow toward the rounded tip, rarely septate, not branched, not geniculate, straight to undulant, 2–4 \times 10–40 μ ; conidia subhyaline to very pale olivaceous, narrowly obclavate, or sometimes cylindrical, straight to curved, indistinctly multiseptate, base short obconically truncate, tip subacute or conical, 2–4 \times 20–75 μ .

On leaves of *Holarrhena antidysentrica* Wall., Balehonnur, Mysore, 29–4–1945, leg. M. J. Thirumalachar.

CERCOSPORA IXORAE Yamamoto. Jour. Soc. Trop. Agr. 6: 602. 1934. Reprint: Phytopath. Lab. Taihoku Imp. Univ. Contrib. 28: 602. 1934.

On leaves of *Ixora parviflora* Vahl., Channapatna, Mysore, 4–6–1944, Nandi Hills, 15–3–1945, leg. M. J. Thirumalachar.

CERCOSPORA JASMINICOLA Muller & Chupp. Arch. Inst. Biol. Veg. Rio de Janeiro 3: 93. 1936.

On leaves of *Jasminum rigidum* Zenk., Bangalore, 22–8–1944, and Nandi Hills, 15–3–1945, leg. M. J. Thirumalachar.

CERCOSPORA KAKI Ell. & Ev. Jour. Mycol. 3: 17. 1887.

On leaves of *Diospyros tupru* Buch. Ham., Bangalore, 15–8–1945, Nandi Hills, 15–3–1945, leg. M. J. Thirumalachar. The leaves of *Diospyros tupru* are often made use of for making native cigarettes. Severe infection by *Cercospora* often results in defoliation.

Cercospora Lettsomiae sp. nov.

Maculae angulares, 2 mm. in diam. usque in areas magnas coalescentes; emarginatae, fuligineae demum fere atrae, infra aliquantum pallidiores; fructificatio praecipue hypophylla; stromata nulla vel cellulis paucis flavo-brunneis composita, conidiophora fasciculata ex hyphis divergentibus 5–20 consistentia, pallide usque pallidissime olivaceo-brunnea, colore uniformia, latitudine irregularia, 0–3-septata, varie curvata vel flexuosa, rare geniculata, interdum ramosa, apice obtuso usque conico, 3–5 \times 10–35 μ ; conidia subhyalina, usque dilute olivacea, cylindrico-obclavata vel brevissima cylindrica, recta usque 1–9-septata, basi obconica, apice obtuso, 3–4.5 \times 15–75 μ .

Leaf spots angular, 2 mm. in diam. to large coalescing areas, no distinct border, fuliginous to almost black, somewhat paler on the

lower surface; fruiting chiefly hypophyllous; stromata none or composed of few yellowish brown cells; conidiophores divergent fascicles of 5-20, pale to very pale olivaceous brown, uniform in color, irregular in width, 0-3-septate, variously curved or bent, rarely geniculate, occasionally branched, blunt to conical apex, $3-5 \times 10-35 \mu$; conidia subhyaline to faintly olivaceous, cylindro-obclavate or shortest ones cylindrical, straight to curved, 1-9-septate, base obconical, tip obtuse, $3-4.5 \times 15-75 \mu$.

On leaves of *Lettsomia elliptica* Wight., Bangalore, 20-12-1945, leg. M. J. Thirumalachar.

CERCOSPORA MALI Ell. & Ev. Jour. Mycol. 4: 116. 1888.

C. Piricola Sawada, Jour. Formosan Nat. Hist. Soc. 17: 3. 1914.

C. minima Tracy & Earle, Bull. Torrey Bot. Club 23: 206. 1896.

On leaves of *Pyrus malus*, Bangalore, 15-8-1945, leg. M. J. Thirumalachar.

Cercospora mysorensis sp. nov.

Maculae subcirculares usque angulares, 1-4 mm. in diam., obscure brunneae, interdum linea elevata marginali et halone flavida vel aurantiaca cinctae; fructificatio amphigena: stromata carentia vel cellulis paucis compositis; caespituli nulli vel hyphis 2-10 divergentibus consistentes; conidiophora pallide vel perpallide fuliginea, colore uniformia, latitudine aliquantum irregularia, 1-7-septata, recta undulata curvatave, ramosa praecipue cum non fasciculata, rare geniculata, apice obtusa rotundata, $4-6 \times 20-150 \mu$; conidia subhyalina usque pallide fuliginea plerumque obclavata sed interdum distincte cylindrica, recta vel curvata, 1-7-septata, basi longe obconice truncata, apice plerumque obtuso, $4-6 \times 20-80 \mu$.

Leaf spots subcircular to angular, 1-4 mm. in diam., dull brown, sometimes surrounded by a raised line border and a yellowish to orange halo; fruiting amphigenous; stromata none or a few dark cells; non-fasciculate or fascicles of 2-10 divergent stalks; conidiophores pale to very pale fuliginous, uniform in color, somewhat irregular in width, 1-7-septate, straight to undulate or curved, branched especially when non-fasciculate, rarely geniculate, bluntly rounded tip, $4-6 \times 20-150 \mu$; conidia subhyaline to pale fuliginous, mostly obclavate but occasionally distinctly cylindrical, straight to curved, 1-7-septate, base long, obconically truncate, tip usually obtuse, $4-6 \times 20-80 \mu$.

On the leaves of *Pouzolzia bennettiana* Wight., Nandi Hills, 17-12-1945, leg. M. J. Thirumalachar. The wide conidiophores and conidia separate this species from others with colored conidia on

the Urticaceae. *Cercospora Pouzolziae* Syd. recorded from Transvaal, Africa, is a different species.

CERCOSPORA NERII-INDICI Yamamoto. Jour. Soc. Trop. Agr. 6: 605. 1934.

On leaves of *Nerium Oleander* L., Tirumalai, Tirupati, 10-1-1946, leg. M. J. Thirumalachar.

CERCOSPORA NYMPHAEACEA Cke. & Ell. Grevillea 6: 89. 1898.

Cercospora exotica Ell. & Ev., Proc. Acad. Nat. Sci. Phila. 45: 463. 1893.

C. Nelumbonis Tharp, Mycologia 9: 111. 1917.

Cercospora Panacis sp. nov.

Maculae circulares, usque 20 mm. in diam., alutaceae, usque fuscae, generaliter margine obscuriori e linea angusta elevata delimitatae, fructificatio plerumque hypophylla; stromata fusca, subglobosa, 15-40 μ in diam.; caespituli plerumque densi, paulo compacti, conidiophora pallide vel pallidissime olivaceo-brunnea, apicem rotundatum conicum versus pallescentia et angustiora, rare geniculata, non ramosa, 2-3.5 \times 10-30 μ ; conidia subhyalina usque pallidissime olivacea, anguste obclavata usque linearia, recta vel leniter curvata, indistincte 1-5-septata, basi obconice truncata, apice obtuso usque conico, 2-3.5 \times 15-65 μ .

Leaf spots circular, up to 20 mm. in diam., tan to medium dark brown, usually with a darker margin which is separated from the remainder of the lesion by a narrow raised line; fruiting chiefly hypophyllous, stromata dark brown, subglobular, 15-40 μ in diam.; fascicles mostly dense, fairly compact; conidiophores pale to very pale olivaceous brown, paler and narrower towards the rounded to conical tip, rarely geniculate, not branched, 2-3.5 \times 10-30 μ ; conidia subhyaline to pale olivaceous, narrowly obclavate to linear, straight to slightly curved, indistinct 1-5-septate, base obconically truncate, tip obtuse to conical 2-3.5 \times 15-65 μ .

On leaves of *Panax fruticosum* L., Bangalore, 5-8-1944, leg. M. J. Thirumalachar.

Cercospora Paramignya sp. nov.

Maculae circulares, 4-12 mm. in diam., centro griseae vel fere atrae e fructificationibus densis atro-punctatae, margine lato albescenti vel alutaceo cinctae, fructificatio plerumque epiphylla; stromata subatra, subcircularis, 30-100 μ in diam., caespituli densissimi, compacti; conidiophora saepe solum e cellulis peripheralibus stromatis consistentia vel interdum usque 2-4 \times 10-

25 μ elongata; non septati, non geniculati, non ramosi, apice fere hyalino; conidia anguste cylindrica, subhyalina, in massa olivacea, indistincte multiseptata, recta vel valde curvata, basi longe obconice truncata, apice subacuto, 1.5-3 \times 25-100 μ .

Leaf spots circular, 4-12 mm. in diam., center gray or almost black with the closely stipple-like fruiting bodies, wide, blanched or tan margin; fruiting chiefly epiphyllous; stromata almost black, subcircular, 30-100 μ in diam.; fascicles very dense, compact; conidiophores often merely peripheral cells on the stroma or occasionally elongated to 2-4 \times 10-25 μ , not septate, not geniculate, not branched, tip almost hyaline; conidia narrowly cylindrical, subhyaline, in mass olivaceous, indistinctly multiseptate, straight to strongly curved, long obconically truncate base, subacute tip, 1.5-3 \times 25-100 μ .

On leaves of *Paramignya* sp., Balehonnur, Mysore, 24-4-1945, leg. M. J. Thirumalachar.

***Cercospora petila* sp. nov.**

Maculae atrobadae usque fere atrae, subcirculares usque irregulares, 2-6 mm. in diam., interdum zona rosea circumdatae; fructificatio plerumque epiphylla; stromata subglobosa, obscura, 20-40 μ in diam., caespituli densi, non compacti; conidiophora pallide usque pallidissime olivaceo-brunnea, apicem anguste rotundatam conicamve versus pallescentia et angustioria, recta usque curvata vel undulata, rare septata, non geniculata, non ramosa, 2-3.5 \times 5-25 μ ; conidia subhyalina vel dilute olivacea, anguste cylindrica, interdum apicem versus leniter attenuata, recta vel curvata, indistincte 1-7-septata, basi rotundata usque subtruncata, apice obtuso usque conico, 2-3.5 \times 30-75 μ .

Leaf spots dark reddish brown to almost black, subcircular to irregular, 2-6 mm. in diam., occasionally surrounded by an old-rose colored zone; fruiting chiefly epiphyllous, stromata subglobular, dark, 20-40 μ in diam.; fascicles dense, not compact; conidiophores pale to very pale olivaceous brown, paler and narrower toward the narrowly rounded or conical apex, straight to curved or undulate, rarely septate, not geniculate, not branched, 2-3.5 \times 5-25 μ ; conidia subhyaline to faintly olivaceous, narrowly cylindrical, sometimes slightly attenuated toward the tip, straight to curved, indistinctly 1-7-septate, base rounded to subtruncate, tip blunt to conical, 2-3.5 \times 30-75 μ .

On the leaves of *Euphorbia pulcherrima* R. Graham (*Poinsettia pulcherrima* L.), Bangalore, 11-11-1945, leg. M. J. Thirumalachar.

CERCOSPORA PUNICAE P. Henn. Bot. Jahrb. von Engler 37: 165. 1906.

On the leaves of *Punica granatum* L., Bangalore, 12-1-1946, Nandi Hills, 28-12-1945, leg. M. J. Thirumalachar.

CERCOSPORA RUBI Sacc. Nuov. Giorn. Bot. Ital. 8: 188. 1876.

Cercospora Septorioides Ell. & Ev., Field Columb. Mus. Bot. Ser. 1: 94. 1896.

C. garbiniana Massalongo, Atti Mem. Acad. Agr. Sci. Lett. Verona Ser. 4, 3: 147. 1902.

On the leaves of *Rubus vulgaris* L., Kemmangundi, Mysore, 9-10-1945, leg. M. J. Thirumalachar.

***Cercospora Shoreae* sp. nov.**

Maculae irregulares, 3-10 mm. in diam., obscure griseae, linea pallide brunnea marginatae; fructificatio amphigena, plerumque epiphylla; stromata fusca, globosa, 15-35 μ ; conidiophora singulatim vel e stromate in fasciculis patentibus, ad apicem obtuse rotundatam angustatis oriunda; 0-2-septata, curvata vel undulata, non ramosa, rare geniculata, 2-4 \times 15-40 μ ; conidia pallide olivacea, anguste obclavata usque fere linearia, recta vel leniter curvata, indistincte 1-5-septata, basi rotundata usque subtruncata, apice obtuso usque conico, 2-4 \times 15-60 μ .

Leaf spots irregular, 3-10 mm. in diam., dull gray, pale brown line margin; fruiting amphigenous but chiefly epiphyllous; stromata dark brown, globular, 15-35 μ ; conidiophores arising singly from the stromata or in spreading fascicles of 2-15, pale olivaceous brown, uniform in color but slightly narrower toward the bluntly rounded tip, 0-2-septate, curved to undulate, not branched, rarely geniculate, 2-4 \times 15-40 μ ; conidia pale olivaceous, narrowly obclavate to almost linear, straight to slightly curved, indistinctly 1-5-septate, base rounded to subtruncate, tip blunt to conical, 2-4 \times 15-60 μ .

On the leaves of *Shorea talura* Roxb., Bangalore, 6-7-1944, leg. M. J. Thirumalachar. This is a new host family for *Cercospora*.

CERCOSPORA SOJINA Hara. Nogyo Sekai, Tokyo 9: 28. 1915.

Cercospora daizu Miura, Manchurian R. R. Agr. Expt. Sta. Bull. 11: 25. 1920.

On the leaves of *Glycine javanica* L., Bangalore, 15-8-1945, leg. Thirumalachar.

CERCOSPORA SORGHII E. & E. Jour. Mycol. 3: 15. 1887.

C. Sorghi var. *Maydis* E. & E. (Langlois Collection No. 613).

On *Amphilophis pertusa* Stapf. (*Andropogon pertusus*), Bangalore, 22-12-1944, leg. M. J. Thirumalachar.

CERCOSPORA SUBSESSILIS H. & P. Syd. Ann. Mycol. 11: 329. 1913.

Cercoseptoria domingensis Ciferri, Ann. Mycol. 36: 231. 1938.

On leaves of *Azadirachta indica* A. Juss.

CERCOSPORA TECTONIAE Stevens Bernice P. Bishop Mus. Bull. 19: 155. 1925.

On *Tectona grandis* L., 30-11-1945, Bangalore, leg. M. J. Thirumalachar.

CERCOSPORA TINOSPORAE H. & P. Syd. Ann. Mycol. 14: 372. 1916.

On the leaves of *Tinospora cordifolia* Miers., Bangalore, 7-10-1945, leg. M. J. Thirumalachar.

Cercospora Waltheriae sp. nov.

Maculae primum indistinctae, demum badiae, angulares, 0.5-5 mm. in diam. vel coalescentes et partem grandem laminae occupantes, interdum aridae dehiscentes; fructificatio in areis oppositis in superficiem inferiorem effusa, dilute griseola usque indistincte olivacea; stromata globosa, pallide brunnea, 20-60 μ in diam.; caespituli densi; conidiophora subhyalina usque pallide olivaceo-brunnea, recta curvatave, non vel rare septata, plerumque non geniculata, non ramosa, apice obtuse rotundato, 2-3.5 \times 5-25 μ , vel longioria cum conidia persistent; conidia subhyalina usque pallidissime olivacea, obclavata usque fere linearia, indistincte multiseptata, recta vel curvata, basi obconice truncata usque rotundata, apice subacuto usque obtuso, 2-4 \times 35-150 μ .

Leaf spots at first indistinct, then becoming reddish brown, angular, 0.5-5 mm. in diam., or coalescing into large part of leaf surface, when dry sometimes dehiscent; fruiting or corresponding areas of the lower surface, effuse, faintly grayish to distinctly olivaceous; stromata globular, pale brown, 20-60 μ in diam.; fascicles dense; conidiophores subhyaline to very pale brown, straight to curved, not or rarely septate, mostly not geniculate, not branched, bluntly rounded tip, 2-3.5 \times 5-25 μ , or when conidia are persistent appearing much longer; conidia subhyaline to very pale olivaceous, obclavate to almost linear, indistinctly multiseptate, straight to blunt, curved, base obconically truncate to rounded, tip subacute to blunt, 2-4 \times 35-150 μ .

On the leaves of *Waltheria indica* L., Bangalore, 2-9-1945, leg. M. J. Thirumalachar. The species differs from *C. Melochiae* recorded on the same host.

***Cercospora Wrightiae* sp. nov.**

Maculae circulares, pallide brunneae usque sordide albae, margine angusto atrobadio cinctae, 0.5-4 mm. in diam.; fructificatio amphigena, stromata subglobosa fusca, 25-60 μ ; caespituli densi, compacti, conidiophora pallide usque moderate olivaceo-brunnea, apicem obtuse rotundatum versus pallescentia et angustioria, sparse septata, non ramosa, rare geniculata, recta vel curvata vel flexuosa, 4-6 \times 10-35 μ ; conidia pallide usque moderate olivacea, obclavata, recta usque leniter curvata, 1-9-septata, basi longe obconice truncata, apice obtuso, 3-5.5 \times 20-65 μ .

Leaf spots circular, pale brown to dingy white, narrow dark reddish brown margin, 0.5-4 mm. in diam.; fruiting amphigenous; stromata subglobular, dark brown, 25-60 μ ; fascicles dense, fairly compact; conidiophores pale to medium olivaceous brown, paler and narrower towards the bluntly rounded tip, sparingly septate, not branched, rarely geniculate, straight to curved or bent, 4-6 \times 10-35 μ ; conidia pale to medium olivaceous, obclavate, straight to slightly curved, 1-9-septate, long obconically truncate base, obtuse tip, 3-5.5 \times 20-65 μ .

On leaves of *Wrightia tinctora* R. Br., Bangalore, 26-12-1945. leg. M. J. Thirumalachar.

CERCOSPORA ZIZYPHI Petch. Ann. Roy. Bot. Gard. Peradeniya Parti 5.4: 306. 1909.

On the leaves of *Zizyphus oenoplia* Mill., Kemmangundi, Mysore, 8-10-1945, leg. M. J. Thirumalachar.

Grateful thanks are due to Dr. Edith K. Cash, Associate Mycologist, U.S.D.A., Beltsville, Maryland, for very kind help in rendering the diagnosis of the new species into Latin. The senior author is deeply indebted to Dr. James G. Dickson, Professor of Plant Pathology, University of Wisconsin, for the benefit of many valuable suggestions.

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PRODUCTION OF ANTIBIOTICS BY SPECIES OF MYROTHECIUM

P. W. BRIAN, H. G. HEMMING AND E. G. JEFFERYS

White and Downing (13) have recently shown that the fungus known as *Metarrhizium glutinosum* Pope (9), widely used in recent years in work on microbiological decomposition of cellulose fabrics, is identical with the well established species *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr. A strain of "*Metarrhizium glutinosum*" has been shown (1, 4) to produce a fungistatic antibiotic, isolated in pure form and named glutinosin, and a volatile highly dermatitic substance, not as yet isolated. It was therefore considered necessary, as was indeed suggested by White and Downing, to examine strains of several species of *Myrothecium* for antibiotic activity, more particularly since strains of several species of *Metarrhizium* had shown no such activity.

ORIGIN OF CULTURES

The cultures examined can all be referred to one or other of the three species described by Preston (11) as *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr., *Myrothecium roridum* Tode ex Fr. and *Myrothecium inundatum* Tode ex Fr. They are listed below with their accession numbers in the culture collection at Butterwick Research Laboratories, Welwyn, England.

No. 173: *Myrothecium verrucaria*: received as *Metarrhizium glutinosum* (U.S.D.A. 1334.2), originally collected from deteriorated baled cotton (7). No. 372: *Myrothecium verrucaria*: received from Centraalbureau voor Schimmelcultures, Baarn, Holland, as *Trichurus terrophilus* Swift & Povah, this culture is almost identical in all characters with No. 173 and in no way corresponds with the original description of *Trichurus terrophilus* (12). No. 402: *Myrothecium verrucaria*: received from N. C. Preston, originally collected by G. R. Bates from washings of citrus fruits in S. Rhodesia (11). No. 401: *Myrothecium roridum*: received from

N. C. Preston, originally collected by him (10) on *Viola*. No. 433: *Myrothecium roridum*: received from F. T. Brooks, originally collected by him (5) on *Lupinus ornatus*. No. 434: *Myrothecium roridum*: received from F. T. Brooks, originally collected by him (5) on *Antirrhinum*. No. 435: *Myrothecium roridum*: received from F. T. Brooks, originally collected by him (5) on potato haulm. No. 436: *Myrothecium inundatum*: received from N. C. Preston. This isolate (Herb. I.M.I. 5605) was originally collected on *Russula adusta*.

Of these, *Myrothecium inundatum* is quite distinct in its cultural characters from the others and more particularly in its much smaller conidia (ca. $4\ \mu$ in length). The cultural appearance of *Myrothecium verrucaria* and *Myrothecium roridum* varies considerably between strains of the same species and the two species cannot be distinguished by any macroscopic culture characteristics. *M. roridum* No. 435 was widely different in cultural appearance from the other strains of *M. roridum* and *M. verrucaria*, being much less vigorous in growth and producing, on Czapek-Dox agar, closely woven pink mycelium instead of the fluffy white mycelium characteristic of the other strains. The only character by which the strains of *M. verrucaria* and *M. roridum* can be distinguished, as has been noted both by White & Downing (13) and by Preston (11), is the spore shape, that of *M. verrucaria* being lemon-shaped and that of *M. roridum* cylindric.

ANTAGONISM IN AGAR CULTURE

(1) *Reversed agar culture test*. This technique has been described in detail by Jefferys (8). It involves growing the fungus to be examined on a suitable agar medium, in this case Czapek-Dox agar, for three days, reversing the agar and inoculating the exposed reverse agar surface with conidia of *Botrytis allii*, the germination and growth of which are then observed with a low-power microscope. Results obtained with the strains of *Myrothecium* by this method are shown in Table I.

(2) *Streak test*. In this test, previously described by Brian & Hemming (3), plates of an agar medium suitable for growth of both bacteria and fungi are streaked with spores of the fungi to be examined, elongated colonies being produced after 3 or 4 days' incu-

bation at 25° C. The test organisms (*Endomycopsis albicans*, *Staphylococcus aureus* and *Salmonella typhi*) are then streaked at right angles to the mold colony and, after a further 24 hours' incubation at 37° C., inhibition is assessed in arbitrary units. Results of this test also are shown in Table I.

TABLE I
ANTAGONISM OF STRAINS OF *Myrothecium* SPP. TO FUNGI AND BACTERIA

Culture No.	Species	Reversed Agar Test <i>Botrytis allii</i>	Streak Test		
			<i>Endomycopsis</i>	<i>Staphylococcus</i>	<i>Salmonella</i>
173	<i>verrucaria</i>	++	++	—	—
372	<i>verrucaria</i>	++	++	—	(+)
402	<i>verrucaria</i>	++	(+)	+	—
401	<i>roridum</i>	++	—	—	—
433	<i>roridum</i>	++	—	—	—
434	<i>roridum</i>	++	—	—	—
435	<i>roridum</i>	+	—	—	—
436	<i>inundatum</i>	—	—	++	—

++ marked inhibition; + moderate inhibition; (+) doubtful inhibition; — no inhibition.

It will be seen that all strains of *Myrothecium verrucaria* and *Myrothecium roridum* produce a substance inhibitory to *Botrytis allii*, whereas *Endomycopsis*, which has been found by experience to be a somewhat more resistant mold, is inhibited only by *Myrothecium verrucaria*. Indications of possible slight antibacterial activity are shown by two strains of *M. verrucaria*. The most significant feature of these results is undoubtedly the antifungal activity of all strains of *M. verrucaria* and *M. roridum*, and the differences between the two species are probably differences of degree only, as results presented later also indicate. *Myrothecium inundatum* is in a class by itself, showing no antifungal activity but showing a well-marked inhibition of *Staphylococcus aureus*. The antibacterial activity of this fungus will be further investigated.

FUNGISTATIC ACTIVITY OF LIQUID CULTURES

Cultures were grown on 250 ml. quantities of various media in "Glaxo" culture vessels for twenty-one days and assayed for antifungal activity at intervals. The method of assay, based on a ger-

mination test with conidia of *Botrytis allii*, has been previously described (2); activity is expressed in BA units per ml. The media had a common basal composition (dextrose 50.0 g., KH_2PO_4 1.0 g., $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g., minor element concentrate (1) 1.0 ml., distilled water 1,000 ml.), and varied in the nitrogen source as follows, the quantity of nitrogen source added being in each case sufficient to provide nitrogen equivalent to 2.3 g. KNO_3 per liter:

Medium N.....	potassium nitrate
Medium AS.....	ammonium sulphate
Medium AS + malate.....	as AS + 0.5 per cent malic acid
Medium A.....	ammonium tartrate

The media were in each case adjusted to pH 4.5 with potassium hydroxide or hydrochloric acid.

The highest assays recorded in each medium with each culture are recorded in Table II.

TABLE II
HIGHEST ASSAYS OF FUNGISTATIC ACTIVITY RECORDED DURING
21 DAYS' INCUBATION AT 25° C.

Culture No.	Species	Highest Assay (BA units/ml.) Recorded			
		N	AS	AS+Malate	A
173	<i>verrucaria</i>	64	32	256	128
372	<i>verrucaria</i>	24	128	256	128
402	<i>verrucaria</i>	64	4	32	128
401	<i>roridum</i>	24	—	64	128
433	<i>roridum</i>	32	—	64	24
434	<i>roridum</i>	16	8	48	32
435	<i>roridum</i>	8	—	8	—
436	<i>inundatum</i>	—	—	—	—

It will be seen that all strains of *Myrothecium verrucaria* and *Myrothecium roridum* produced fungistatic culture filtrates, those of *M. verrucaria* being more active than all but one (No. 401) of the *M. roridum* cultures. *M. roridum* No. 435 was much less active than the other cultures; this strain, as has already been mentioned, was different in gross-culture appearance from the other strains. *Myrothecium inundatum* was again found to be fungistatically inactive.

It remains to be confirmed by chemical work, now in hand, whether the fungistatic substance produced by the various strains

of *Myrothecium verrucaria* and *Myrothecium roridum* is glutinosin (4).

DERMATITIC ACTIVITY OF CULTURE FILTRATES

Dermatitic or vesicant activity was determined by a simple patch test on the forearms of two subjects. Small circles of filter paper were soaked in culture filtrate (in each case the filtrate from Medium AS + malate was used) and strapped on the forearm for 48 hours, when the plaster was removed and the underlying skin examined. A clear positive dermatitic response was shown to all *Myrothecium verrucaria* filtrates and to all *Myrothecium roridum* filtrates except that from No. 435, which has also been shown to be aberrant in other respects. The filtrate from *Myrothecium inundatum* was completely inactive. The specificity of this reaction is remarkable and, in spite of all the large scale culture work with molds carried out in recent years, the only similar record is that of certain Russian workers (6) with a strain of *Stachybotrys alternans* Bonord.

SUMMARY

The mold previously known as *Metarrhizium glutinosum* Pope has recently been shown to be identical with the well-established species *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr. A strain of "*Metarrhizium glutinosum*," previously shown to produce, in the course of its metabolism, two biologically active substances, one highly fungistatic (glutinosin) and the other causing severe dermatitis, has been compared with several strains of *Myrothecium verrucaria*, *Myrothecium roridum*, and *Myrothecium inundatum*. The strains of *Myrothecium verrucaria* and *Myrothecium roridum* behaved similarly, all producing a fungistatic substance (not yet shown to be chemically identical with glutinosin) and all except one strain of *Myrothecium roridum* producing the dermatitic substance. This result emphasizes the very close relationship between *Myrothecium roridum* and *Myrothecium verrucaria*. A single strain of *Myrothecium inundatum* examined did not produce either the fungistatic or the dermatitic substance.

ACKNOWLEDGMENTS

The authors are greatly indebted to Dr. W. Lawrence White for communicating to them his conclusions concerning the identity of "*Metarrhizium glutinosum*" in advance of his publication. They are indebted for cultures to Professor F. T. Brooks, Mr. N. C. Preston and Prof. Dr. Johanna Westerdijk.

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MUTUAL ANTAGONISM BETWEEN PATHOGENIC FUNGI. INHIBITION OF DIMORPHISM IN *CANDIDA ALBICANS*

OTIS F. JILLSON ¹ AND WALTER J. NICKERSON ²

(WITH 2 FIGURES)

Clinically, infection by two or more pathogenic fungi in the same person is of rare occurrence. Muskatblit (1941) reported six cases of double infection, and reviewed thirty-six cases from the literature. He concluded that the rarity of combined fungous infections suggests the presence of a certain degree of immunity created by the first invader, which protects the individual from a superimposed infection with another fungus. Lewis and Hopper (1943) reported on twenty-three cases of multiple fungous infections, of which nine (five being doubtful) were considered to have combined infections in which the species of fungi were working together. Two of these cases were combined infections of *T. rubrum* and *C. albicans*. *T. rubrum* did not appear until cultures were a month old. In a later article, Muskatblit (1946) presented a case of dermatophytosis, of the sole of the foot, resulting from an infection with *Trichophyton mentagrophytes* (*interdigitale*) and *T. rubrum* (*purpureum*). Both fungi were isolated and grown in culture. The clinical picture, of vesicles and good response to therapy, was that of a fungous infection caused solely by *T. mentagrophytes*—rather than the dry, scaly, infiltrated, therapeutic-resistant type of lesion caused by *T. rubrum*. In addition to his earlier conclusions, Muskatblit suggested it was possible that *T. mentagrophytes* acted as an antagonist of *T. rubrum*. Since one

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usually isolates pure cultures of *Candida albicans* from lesions produced by this fungus, Lewis and Hopper (1943) observed that there appears to be an inhibitory effect on the growth of other microorganisms in the presence of *C. albicans*.

Our own clinical observations certainly tended to confirm the extreme rarity of mixed fungous skin infections. To see if there were any mutually antagonistic effects observable with pathogenic fungi *in vitro*, we studied two-membered pure cultures of *Trichophyton rubrum* and *Candida albicans*. Notable effects of this method of culturing these two fungi were seen on the growth rates and coloration of *T. rubrum*, and the formation of mycelia by *C. albicans*.

MATERIALS AND METHODS

The following fungi were used:³

Candida albicans, No. 624, isolated from a case with chronic paronychia with nail involvement, at the Dermatologic Clinic, Boston City Hospital.

Candida albicans, No. 732, similar source, a second case.

Candida albicans, No. 810, isolated from a case of Perlèche.

Trichophyton rubrum (*purpureum*), No. 706, isolated from the finger nails in a case of tinea unguium.

Stock cultures of strains of *C. albicans* were maintained on slants of Difco-corn meal agar with an additional 0.5 per cent of Bacto-agar incorporated. *T. rubrum* was maintained on Difco-Sabouraud's agar. Stock cultures were incubated at room temperature. In some experiments media buffered to a definite pH were employed. For these, McIlwain's NaOH-KH₂PO₄ buffer series was used.

All pH determinations were made with a Beckman glass electrode. In one series, indicator dyes were incorporated into the medium to permit visualization of the course of pH change during growth in the single and two-membered cultures. The dyes used were from a set marketed under the name "universal indicator."

³ The assistance of Miss Louise A. Kelley with isolation and identification of the fungi is appreciated.

GROWTHS AND COLORATION OF TRICHOPHYTON RUBRUM

When *Candida albicans* and *Trichophyton rubrum* are grown together on an agar slant in a two-membered culture, mutually antagonistic effects can be observed. The wine-red color of *T. rubrum* growing singly on corn meal agar is replaced by a yellow-brown color when growing in the presence of *C. albicans*. As we show later, this color change is a result of the acid pH of the two-membered culture. The pigment of *T. rubrum* is a pH indicator. The growth of both fungi is inhibited in the mixed culture when compared with the amount of growth obtained in single-membered cultures. Mycelial development, clearly evident in the pure culture of *C. albicans*, is completely inhibited in the two-membered culture.

It was observed that cultures of *C. albicans* reached a pH of 3.0-3.2 within twenty-four hours when grown in a liquid medium with agitation. We considered the acid-producing abilities of this yeast might be responsible for so lowering the pH in the two-membered culture that the internal H-ion concentration of the cells of *T. rubrum* (reflecting the hydrogen ion concentration of their environment) would result in the pigment existing in a yellow-brown (acid) color.⁴

Accordingly, a series of buffered corn meal agar media was inoculated with *T. rubrum*. Good growth was obtained within the pH range employed (4.5-8.0). At pH 4.5, cultures of *T. rubrum* were yellow-brown, closely approximating the color obtained in the two-membered cultures; at pH 8.0 the coloration developed was wine-red.

Irrespective of the pH, however, the color appearing initially in cultures of *T. rubrum* on the buffered corn meal agar we employed was yellow-brown; this color then persisted or turned red in a short time depending on the pH. On Difco-Sabouraud's agar (not specially buffered) the pigment was red initially and remained so as the culture developed.

⁴ Experiments *in vitro* on the pigment extracted from *T. rubrum* (and other dermatophytes) have shown it to exhibit a red color in alkaline ranges and a yellow-brown color in acid ranges. It has been suggested (Nickerson 1947) that one pigment (existing in a state determined by the intracellular physico-chemical environment) would suffice to account for the known facts relating to coloration of dermatophytes in culture.

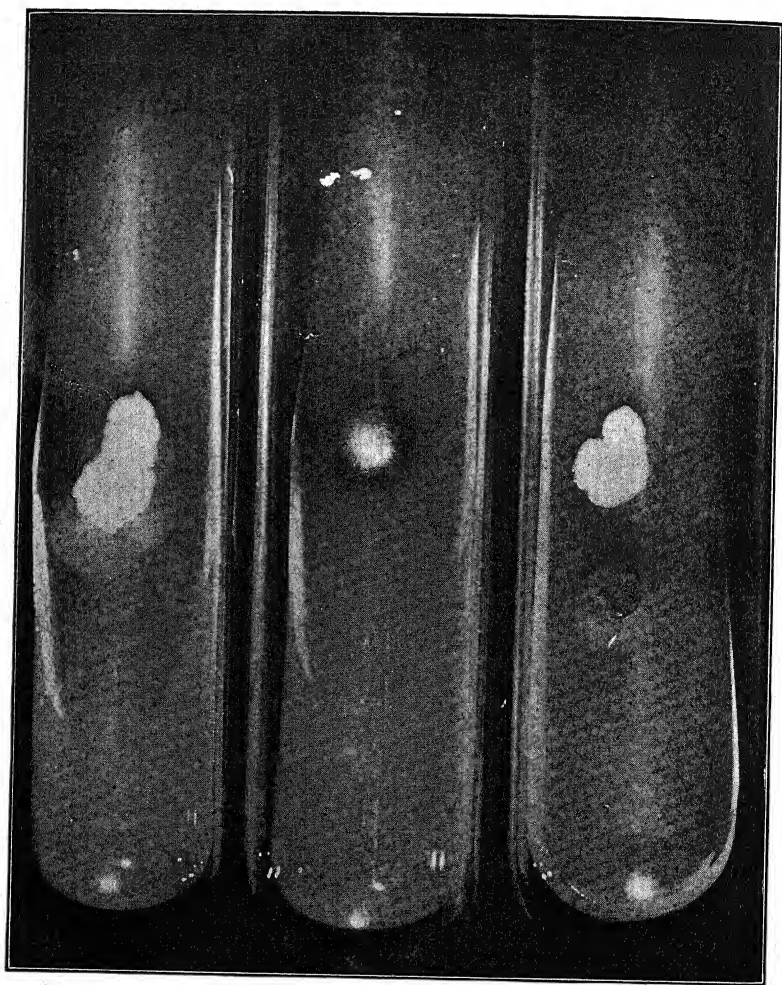


FIG. 1. *Candida albicans* and *Trichophyton rubrum*.

Inhibition of growth of *T. rubrum* in the two-membered culture is also evident (FIG. 1). This effect of the presence of *C. albicans* may have resulted from the diffusion of metabolic products through the medium (the two organisms were not perceptibly in direct contact with each other). To examine this hypothesis *C. albicans* was grown in pure culture in a liquid medium⁵ with aeration for

⁵ Composition: 2.0 g. glucose, 1.0 g. glycine, 0.1 g. Difco-yeast extract, and 100 ml. distilled water.

twenty-four hours; a very heavy growth was obtained. A culture was then passed through a Corning fritted glass filter (porosity UF) to obtain a sterile filtrate which was distributed in corn meal agar media (cooled to 40° C.) to give a final concentration of 2.0 per cent filtrate by volume. One set of such tubes was autoclaved; a second set was not heated. The tubes were inoculated with *T. rubrum* and incubated at 25° C. The unheated filtrate in 2.0 per cent concentration showed considerable inhibitory effect on the growth of *T. rubrum*, whereas the heated series and controls were practically indistinguishable.

DIMORPHISM OF CANDIDA ALBICANS

One of the most interesting aspects of morphogenesis in the fungi is the conversion of cells from the single-celled, budding, yeast-like form of growth to the filamentous, mycelial pattern. This transition, and the reverse thereof, is a not uncommon phenomenon, occurring among widely diverse members of the fungi. To mention a few examples: (1) *Blastomyces dermatitidis*, *B. brasiliensis*, and *Histoplasma capsulatum* are yeast-like at 37° C., mycelial at 25° C.; (2) *Candida albicans* is usually yeast-like during the first stages of growth on agar media, but may, under some conditions, later develop pseudomycelial and mycelial elements which may terminate with large chlamydo-spores; (3) species of *Mucor*, kept submerged in liquid media by agitation, exhibit a single-celled, yeast-like form of growth and may carry out an alcoholic fermentation of carbohydrates. This list could be greatly expanded. There have been several experimental investigations on methods for obtaining a yeast (*Y*) to mycelial (*M*) conversion ($Y \rightarrow M$) in culture, but the cellular mechanisms involved in such conversions have received comparatively little attention.

Variability in the yeasts producing mycelia (the *Mycotoruloidae*) is well recognized; see Wickerham and Rettger (1939) and Diddens and Lodder (1942) for extensive discussions. Whether or not a given isolate of one of these yeasts will develop true mycelia in addition to pseudomycelia is apparently unpredictable, being the result of the interaction of several known, and probably several unrecognized, variables. We have not concerned

ourselves particularly with this aspect of the problem and have restricted our attention to three strains of *Candida albicans* that readily formed mycelia on corn meal agar⁶ slants (many isolates apparently require more anaerobic conditions).

Our working hypothesis with regard to the suppression of mycelium formation by *C. albicans* observed in the two-membered cultures was that metabolic product(s) of *T. rubrum* diffused through the agar medium and inhibited the development of dimorphism in *Candida*. To examine this hypothesis, sterile filtrates were prepared (as mentioned previously) from cultures of

TABLE 1
EFFECT OF ADDITION OF STERILE FILTRATES FROM *T. rubrum* BROTH CULTURES ON THE $Y \rightarrow M$ CONVERSION IN *C. albicans*

Concentration of Filtrate (Volumes Per cent)	Time of Appearance of $Y \rightarrow M$ (Days)	Rate of Mycelial Growth (Control = 1)
0	4	1
0.5	6	1
1.0	10	<1
2.0	>30	—
2.0 (heated)	9	>1
5.0 (heated)	>30	—

T. rubrum grown for two weeks at 25° C. in 125 ml. Erlenmeyer flasks containing 50 ml. of Difco-malt extract broth (initial pH 5.5). Varying quantities of such a filtrate were added to tubes of sterile corn meal agar cooled almost to solidifying (ca. 40° C.); tubes were shaken briefly after the addition of liquid and then slanted. Amounts of filtrate added were such as to give final concentrations of 0.5, 1.0, 2.0, and 5.0 per cent filtrate by volume. Identical series were prepared which were autoclaved (15 lbs. for 20 mins.)

⁶ Considerable variation among lots of corn meal agar (both Difco and home made media) was observed; an attempt was made to analyze the basis of these differences. High carbohydrate content of the medium has been implicated by many authors as a reason for persistence of *C. albicans* in the yeast phase. While there may be some basis for this opinion, we have prepared different lots of Difco media with approximately identical concentrations of glucose (0.2 per cent by analysis) and observed excellent mycelial development with one lot and none with another. Additions of auxin (indole-3-acetic acid) ranging from 0.001 to 10.0 mg. per liter final concentration to a corn meal agar medium (Difco) prepared from a batch that did not support $Y \rightarrow M$ failed to induce the conversion to *M*. This was the case for a series held at 20° C. and for another held at 25° C.

after addition of the filtrates to the tubes. Tubes in the two series were inoculated with *C. albicans* and incubated at 25° C.

As seen in Table 1, the filtrate from *T. rubrum* possessed marked ability to inhibit mycelial development by *C. albicans*. This property was altered to a considerable extent by heating. The unheated filtrate had very little (and the heated filtrate even less) inhibitory effect on the growth of *C. albicans* as a yeast (FIG. 2). The heated filtrate in 2.0 per cent concentration retarded mycelial development for five days. Once initiated, development of mycelium was relatively more rapid in cultures with heated filtrate (up to 2.0 per cent concentration) than in the controls; chlamydospores were produced as they were in the controls. Unheated filtrate in a concentration as low as 2.0 per cent completely prevented the $Y \rightarrow M$

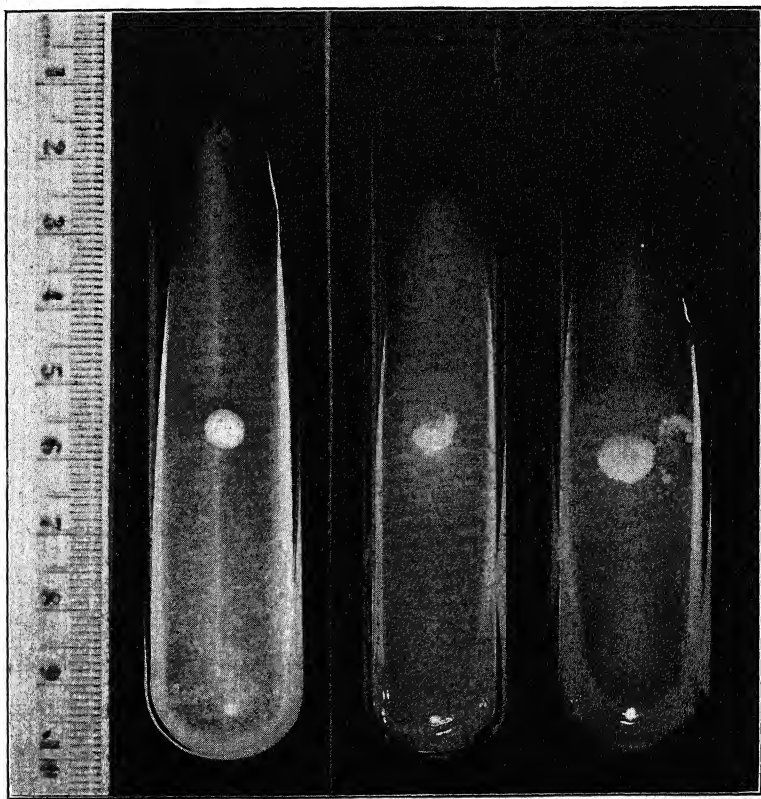


FIG. 2. *Candida albicans* and *Trichophyton rubrum*.

conversion (cultures examined for a thirty day period); only yeast cells were visible microscopically in such cultures; the *Y* cells were apparently normal and exhibited dimorphism when transferred to plain corn meal agar media within the usual time of three to four days.

PRELIMINARY FRACTIONATION OF *T. RUBRUM* FILTRATES

A *T. rubrum* filtrate (prepared as described above) was treated with charcoal (0.2 g. Norite/100 ml. filtrate) at 50° C. with vigorous stirring. The charcoal was removed by filtration through Whatman paper (no. 50) and the filtrate sterilized by passing through a fritted glass filter. This sterile filtrate was tested for activity. The residue from the Norite treatment was eluted with water containing 1.0 per cent acid alcohol by volume. This mixture was filtered and sterilized as just described and the sterile eluate tested for activity. The filtrate from another identical culture of *T. rubrum* was concentrated *in vacuo* to a thick syrup. To this twenty volumes of acetone were added; the acetone-soluble fraction was separated by filtration, evaporated to dryness under reduced pressure, and dissolved in a volume of water equal to the original filtrate. The acetone insoluble material was dissolved in a similar volume of water. Each of these aqueous solutions was sterilized by filtration and tested for activity.

TABLE 2
PRELIMINARY FRACTIONATION OF *T. rubrum* FILTRATE FOR $Y \rightarrow M$
INHIBITORY ACTIVITY

Fraction (All 2 Per cent by Vol.)		Time of Appearance of $Y \rightarrow M$ (Days)
Control		5
Norite adsorbed	heated	10
	unheated	10
Not adsorbed	heated	5
	unheated	7
Acetone soluble	heated	7
	unheated	7
Acetone insoluble	heated	5
	unheated	7

It is evident from Table 2 that much of the mycelium-inhibiting activity of the *T. rubrum* filtrate has been adsorbed by the Norite, and that it can be eluted from the Norite (the eluting agent tested by it-

self was without effect). The Norite-adsorbed fraction is heat stable. Heat stable inhibitory activity is also found in the acetone-soluble fraction. The heat labile inhibitory activity appears to reside in the acetone-insoluble and Norite-non-adsorbed fractions. The inhibitory action of *T. rubrum* on the $Y \rightarrow M$ conversion in *C. albicans* thus appears to be due to two diffusible metabolic products: (1) soluble in water and acetone, heat stable, and adsorbed by Norite from which it can be eluted by dilute aqueous-acid alcohol; (2) water soluble, acetone insoluble, heat labile, and not adsorbed by Norite.

EFFECT OF pH AND NH_4 CONCENTRATION ON DIMORPHISM OF CANDIDA

It is well known that cultures of dermatophytes become alkaline; Goddard (1934) showed this to be a result of the production of ammonia—probably by the oxidative deamination of amino acids. We examined the possibility that pH or the ammonium ion might be involved in the suppression of dimorphism in *C. albicans*. Inoculations onto slants of buffered corn meal agar (prepared as described previously) resulted in mycelial production at all H-ion concentrations (4.5–8.0) within four days; at pH 4.5 mycelial formation was evident in three days.

TABLE 3

EFFECT OF ADDITION OF NH_4Cl ON $Y \rightarrow M$ CONVERSION IN *C. albicans*

Concentration of NH_4Cl (Per cent w/v)	Time of Appearance of $Y \rightarrow M$ (Days)
0.0	4
3.0 (unheated)	>15
3.0 (heated)	>15
1.0 (heated)	>15

Ammonium chloride, added to corn meal agar to give final concentrations of 0.05, 0.1, 0.5, and 1.0 per cent NH_4Cl , resulted in rather erratic appearance of mycelial growth; with most cultures the appearance of *M* was delayed, but in some tubes with the lower concentrations of NH_4Cl the $Y \rightarrow M$ conversion was as rapid as in the controls, and in one experiment the cultures with NH_4Cl (all concentrations) actually appeared a day earlier in the *M* form than did the controls. An additional series with higher concentra-

tions of NH_4Cl was studied (TABLE 3). Consistent inhibition of $Y \rightarrow M$ was obtained with 3 per cent NH_4Cl in the medium.

Ammonia production by *T. rubrum* is very small during the first seven days growth in broth cultures, but increases rapidly thereafter. The change in pH in such cultures, due chiefly to the production of ammonia, is likewise small during the first week of incubation. In the two-membered cultures (FIG. 1) it is clearly evident that the active metabolic products of *T. rubrum* must be produced in sufficient quantity by the fourth or fifth day of incubation (judging from the time of $Y \rightarrow M$ conversion in pure culture) to prevent $Y \rightarrow M$ in *C. albicans*. The total ammonia produced (expressed as NH_4Cl) by *T. rubrum* in broth cultures after thirty days incubation is certainly less than 0.1 per cent. Since our results with such low concentrations of NH_4Cl were inconclusive and, in view of the small quantity of ammonia produced by *T. rubrum* during early stages of growth, it must be concluded that the dimorphism inhibitory activity of *T. rubrum* filtrates cannot be attributed to the NH_4^+ ion or to a change in the pH of the medium.

GENERAL ASPECTS OF $Y \rightarrow M$ DIMORPHISM

Several examples of fungi capable of existing in dimorphic states, as *Y* or *M*, were presented earlier. The phenomenon of dimorphism among microorganisms is quite widespread; thus the prominence of the feature among pathogenic forms may be more apparent than real. In addition to examples given previously, dimorphism is seen among the Protozoa in *Leishmania* and *Histomonas* which are amebic in tissues and flagellate in culture.⁷ The smuts are yeasts in culture and mycelial in their plant hosts.

Among the bacteria the production of a filamentous type of growth can be brought about in a variety of ways (TABLES 4, 5, and 6); Hinshelwood (1946) has discussed the problems at length from a physico-chemical point of view and Lea (1947) from the

⁷ We are indebted to Professor L. R. Cleveland for drawing our attention to this analogous situation among the pathogenic protozoa. It is interesting to see that here too the dimorphism expressed could serve as a basis for classification of the organism in one or another of two large groups—exactly as might be done, in some instances, for the fungi on the basis of a *Y* or an *M* form.

TABLE 4
CONDITIONS FAVORING $Y \rightarrow M$ OR $B \rightarrow F$ CONVERSIONS

Condition	Organism	Remarks	Author
1. Nutritional:			
a. Di- or polysaccharide carbon source	<i>C. albicans</i>		4, 18
b. NH_4^+ nitrogen source	<i>B. lactis aerogenes</i>		9, 10
c. Transfer from NH_4^+ to NO_3^- nitrogen source	<i>E. coli</i> and <i>B. lactis aerogenes</i>		9, 10
2. Specific substances:			
a. Methyl violet	<i>E. typhosum</i>		1
b. Proflavine	<i>B. lactis aerogenes</i>		9, 10
c. <i>m</i> -Cresol	<i>B. lactis aerogenes</i>		9, 10
d. Penicillin	Many Gram - and Gram + bacteria	Effect obtained at 10-30-fold dilutions of bacteriostatic concentrations	5
e. Filtrate from grown cultures of <i>B. lactis aerogenes</i>	<i>B. lactis aerogenes</i>	Shortens lag phase; L-factor favored more than D-factor	9, 10
3. Physical agents:			
a. Ionizing radiations	<i>E. coli</i> , etc.	Usually permanent effect	3, 7, 12, 14, 15, 28
b. Elevated temperature (40° C.)	<i>B. lactis aerogenes</i>	Cells normal at 30° C. even in presence of proflavine or <i>m</i> -cresol	10, 11
c. Lowered temperature (<ca. 30° C.)	<i>Blastomyces dermatitidis</i> and <i>B. braziliensis</i>	Thermal dimorphism	16, 25
d. Osmotic pressure (M/20 NaCl)	<i>B. lactis aerogenes</i>	Enhances the proflavine or <i>m</i> -cresol effect	10, 11

TABLE 5
CONDITIONS INHIBITING $Y \rightarrow M$ OR $B \rightarrow F$ CONVERSIONS

Condition	Organism	Remarks	Author
1. Nutritional:			
a. Amino-acid-nitrogen source	<i>B. lactis aerogenes</i>	Division keeps pace with elongation; only "normal" cells seen	9, 10
b. Dextrose-carbon source	<i>C. albicans</i>		4, 8, 18, 27
c. Adequate aeration	<i>C. albicans</i>	Some strains	4, 8, 27
2. Specific substances:			
a. <i>T. rubrum</i> filtrate	<i>C. albicans</i>	Two fractions (see text)	This paper
3. Physical agents:			
a. Osmotic pressure	<i>B. lactis aerogenes</i>	Except in critical range of M/20 NaCl, osmotic pressures inhibitory	10, 11
b. Elevated temperature (34-37° C.)	<i>Blastomyces dermatitidis</i> <i>B. braziliensis</i>	Thermal dimorphism, apparently independent of nutritional level	16, 25

recent work with ionizing radiations. Hinshelwood cites conditions favoring the formation of abnormally long cells as (a) presence of certain drugs which inhibit division without inhibiting growth to the same extent—for example, Ainley-Walker and Murray (1904) obtained filaments by growing *Bact. typhosum* in the presence of methyl violet and other dyes; and (b) transfer of the cells to an unaccustomed medium to which the growth and division functions adapt themselves at different rates.

Hinshelwood and Lodge (1944) observed very long filaments with *Bact. lactis aerogenes* on transfer from bouillon to a glucose-inorganic salts-ammonium sulphate medium in which the glucose concentration was low (0.19 per cent). This we may designate as a bacterium to filament conversion ($B \rightarrow F$) in analogy to $Y \rightarrow M$ with *Candida*. With a higher glucose concentration (3.85 per cent), the same medium supported a population of only normalized cells. They viewed this as a lack of adaptation of the cells on the transfer from bouillon to the low glucose medium; serial subculture in either glucose medium was accompanied by a gradual disappearance of filamentous cells on inoculation into the dilute glucose medium. Hinshelwood (1946) developed the hypothesis that two separate factors favor elongation (L factor) and division (D factor) respectively, with the former diffusible into the medium. Amino acid media, in contrast to ammonium sulphate media, favor the production of factor D, and $B \rightarrow F$ was never observed in a glucose-asparagine medium. A filtrate from cultures of *Bact. lactis aerogenes* hastened the onset of growth, shortening the lag period, when added to culture media; in some instances cell division was not stimulated correspondingly thus favoring the conversion of $B \rightarrow F$.

From a genetical viewpoint it is clear that the dimorphic phenomena in *C. albicans*, and in some of the other examples discussed (TABLE 4), cannot be considered as mutations. The morphogenetic conversions are not stable and will revert to the original form on the return of the elongated cells to a suitable environment; furthermore, it is apparent in many cases that a high proportion of a population has undergone the $Y \rightarrow M$ or $B \rightarrow F$ conversion. Figure 2 shows the occurrence of $Y \rightarrow M$ from the entire periphery of

the *Y* colony. In other instances it appears probable that $B \rightarrow F$ may result from a mutation as judged by the permanence of *F*.

The mutation-producing action of X-irradiation is well known since the first demonstration by Muller (1927) on *Drosophila*. By such techniques Haberman and Ellsworth (1941) obtained elongated cocci from *Staphylococcus aureus*. Kempster (1917) early observed that some cultures of bacteria that had been X-irradiated might fail to divide but were not dead and would continue to elongate into filamentous elements. Such elongated forms have been subsequently studied by Lea et al. (1937), Witkin (1946) and others. Eisenstark and Clark (1947) examined greatly elongated cells of *Escherichia coli* (X-ray induced) in the electron microscope; they found definite "breaks" in the cell spaced periodically along its length, possibly at the sites where the cell normally would have divided. They advanced the hypothesis that an "enzyme responsible for pinching off the cell wall in normal bacterial fission has been destroyed by rays while other enzyme systems continue to function."

From the recent investigations of Knaysi (1944) and Robinow (1945) it is clear that localized events inside the cell precede the fission of the cell and probably exert the causative effect; neither worker found any indication of cell division by simple constriction.

If we consider the division of cells to be controlled by a single enzyme or enzyme complex (D factor of Hinshelwood) then it

TABLE 6

THEORIES ADVANCED TO EXPLAIN THE $B \rightarrow F$ AND $Y \rightarrow M$ PHENOMENA

Author	Theory
1. Linossier & Roux 1890	"The complexity of form of the thrush parasite (<i>C. albicans</i>) is proportional to the molecular weight of the food elements of the medium (8)."
2. Henrici 1930	Conditions favoring rapid growth of <i>C. albicans</i> cause <i>Y</i> to predominate; less favorable conditions encourage appearance of <i>M</i> form.
3. Hinshelwood 1946	Cell Division (D) and Elongation (L) under control of two independent factors that can get out of balance, as judged by statistical distribution of cell sizes, from various causes.
4. Eisenstark & Clark 1947	A "pinching off" enzyme responsible for cell division is inactivated (by X-rays) while other enzymatic activities are unhampered. (Compatible with the "D factor" of Hinshelwood.)

would follow that substances acting as inhibitors of this system would occasion a lag in division and possibly the appearance of $B \rightarrow F$ and $Y \rightarrow M$. Since inhibition of such an enzyme system would probably not result in rapid death of the cell, we would find an explanation for the reversion of $F \rightarrow B$ and $M \rightarrow Y$ when the inhibitory agent is removed and the cell restored to an environment supporting normal growth. Should the "pinching off" enzyme system be permanently inactivated (and the genic method for its regeneration⁸ abolished) the conversion of $B \rightarrow F$ and $Y \rightarrow M$ would assume a permanent character.

DISCUSSION

The data presented on the *in vitro* effects of association of *Trichophyton rubrum* and *Candida albicans* in two-membered pure cultures indicate the latter exerts a fungistatic action on the growth of *T. rubrum*. Filtrates from liquid cultures of *C. albicans* had a similar action, the activity being destroyed on autoclaving. These findings may, in part, explain the clinical findings that *C. albicans* is usually isolated from lesions as a pure culture. We are not in a position at present to say anything about the pathogenicity of either species after treatment with metabolic products of the other.

Filtrates from broth cultures of *T. rubrum* did not noticeably affect the growth of *C. albicans* as a yeast but completely suppressed the appearance of the normal phenomenon of yeast to mycelium conversion in the three strains studied. In the general aspects of the morphogenetic problem presented as $Y \rightarrow M$ or $B \rightarrow F$ specific instances of interference with morphogenetic pattern are seen in the action of agents promoting cell elongation, possibly through interference with cell division. If this be accomplished, as the accumulated evidence appears to indicate, by the inhibition or total

⁸ McIlwain (1946), in a paper of far-reaching significance, has calculated that some enzymes may exist as only one or a few units in a microbial cell, suggesting that in microorganisms some enzymes may be identical with the respective genes—the enzyme having a dual hereditary and enzymatic function. It is doubtful that the hypothetical "pinching-off" enzyme would be involved in any of the more rapid cell reactions (respiration, protoplasmic synthesis, cell wall elaboration, etc.) that proceed during its inactivation. Therefore, it possibly has a low turnover number and might belong to the group McIlwain regards as existing as one or at most a few units in a cell.

inactivation of a unit enzyme system responsible for cell division, the equivalent action of diverse chemical and physical agents becomes more understandable. Non-appearance of the *M* stage in *C. albicans* (i.e., maintenance of the population in the *Y* state) in a strain where $Y \rightarrow M$ is a normal occurrence in culture is more difficult of interpretation. In general, an environment supporting good growth causes the population to appear only as the *Y* or *B* form (see TABLE 5); to use Hinshelwood's interpretation, the growth and division factors in the cells of the population are well balanced. Since the *T. rubrum* filtrates appeared to be without effect on the growth rate of the *Y* form of *C. albicans* in a good nutrient medium, one might conjecture that the normal occurrence of $Y \rightarrow M$ in *C. albicans* cultures after the third or fourth day is stimulated or occasioned by metabolic products of *C. albicans* and this effect is specifically neutralized by the active *T. rubrum* fractions.

SUMMARY

By means of two-membered pure cultures the pathogenic fungi, *Trichophyton rubrum* and *Candida albicans*, have been shown to exert antagonistic effects on one another *in vitro*. The effects observed were (1) inhibition of growth (marked inhibition of *T. rubrum* by a heat-labile metabolic product of *Candida*), (2) the production of a yellow-brown color by *T. rubrum* in the two-membered cultures as a result of acid production by *Candida* (the pigment of *T. rubrum* is a pH indicator), (3) complete inhibition of mycelial production by *C. albicans*.

The latter phenomenon was studied in some detail. Two metabolic products of *T. rubrum* were shown to inhibit the yeast to mycelial conversion (designated as $Y \rightarrow M$). These products were: (1) soluble in water and in acetone, heat stable, adsorbed by Norite and eluted from it by dilute aqueous acid alcohol, (2) soluble in water, insoluble in acetone, heat labile, not adsorbed by Norite.

Conversion of $Y \rightarrow M$ was independent of pH (4.5–8.0), but was inhibited regularly by high concentrations of NH_4Cl (3 per cent). It was concluded the active fractions of *T. rubrum* filtrates were not the H^+ or NH_4^+ ions.

The general phenomenon of inhibition of cell division without simultaneous inhibition of growth (resulting in the production of elongated cells) is discussed in some detail. The possibility of inhibition or inactivation of a single enzyme complex responsible for cell division is briefly considered.

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EXPLANATION OF FIGURES

FIG. 1. Two-membered pure culture (right) of *Candida albicans* (upper) and *Trichophyton rubrum* (lower). Single-membered cultures of *C. albicans* (left) and *T. rubrum* (center). Note the complete suppression of mycelial development in *C. albicans* in the 2-membered culture. The coloration of *T. rubrum* in the same culture was a burnt orange, while in the single culture (center) it was characteristic wine red. Note differences in size of the two growths.

FIG. 2. Effect of *T. rubrum* filtrates on $Y \rightarrow M$ in *Candida albicans*. 14 day cultures of *C. albicans* on corn meal agar; left, 2 per cent unheated filtrate from broth culture of *T. rubrum*; center, control on plain corn meal agar; right, 2 per cent autoclaved filtrate of *T. rubrum* culture. Complete suppression of mycelia at left; absence of inhibitory effect at right. The authors thank Mr. White for assistance with the photographic work.

NOTES AND BRIEF ARTICLES

A CELLULOSE-DEGRADING GLIOCLADIUM

A *Gliocladium* (identified by K. B. Raper (1) as a member of the *roseum* series) shows considerable ability to degrade cellulose. This organism was isolated from a sedge peat marketed commercially as "Michigan Peat." As far as it has been possible to ascertain, this character has not been previously reported for members of this series.

Using unsized cotton strips and testing for loss of tensile strength with a Scott breaking strength tester, this organism in one month produced degradation to such an extent as to show a loss in tensile strength of 64.7 per cent. Similar results were obtained recently by White (2) at the Biological Laboratories of the Quartermaster Depot in Philadelphia, using another strain (Fla. B-34) of the *G. roseum* series.

This percentage loss was determined by using 3.7 ounce cotton strips 6 inches by $1\frac{3}{16}$ inches. The strips were leached in several changes of distilled water, sterilized by autoclaving and oven dried at 65° C. These strips were then placed in French square bottles containing 35 ml. of Czapek's Mineral Agar composed as follows: NaNO_3 2.0 grams, K_2HPO_4 1.0 gram, MgSO_4 0.5 gram, KCl 0.5 gram, FeSO_4 0.01 gram, agar 15.0 grams, water 1,000 ml. The inoculation was made by pipetting 1 ml. of a heavy spore suspension onto the surface of the strip. Twelve bottles were inoculated and incubated at 30° C. In groups of 4 bottles each, these were harvested at the end of periods of 1, 2 and 4 weeks. A series of 4 uninoculated strips was subjected to all the processing and incubation given the test strips. These strips were used as a control and the standard of tensile strength. After the strips were removed from the bottles they were immersed in formalin for 3 minutes and then washed in running water, oven dried at 65° C. and stored in a desiccator until time for testing. The formalin treatment used here to preclude the possibility of any further growth has been found by Hutchinson (3) to have no appreciable effect on the

tensile strength of such cotton strips. Prior to testing, the strips were hung for 24 hours in a chamber which was maintained at a constant temperature of 21° C. and a relative humidity of 65 per cent. The strips were tested in this chamber on a Scott Break Testing Machine. The average breaking strength for each group was compared with the breaking strength of the average of the control strips. The following table gives the result:

Duration of Growth	Breaking Strength in lbs.				Average, lbs.	Loss of Tensile Strength, Per Cent
	Sample 1	2	3	4		
One week	34	28	32	29	30.7	26.9%
Two weeks	31	24	19	24	24.5	41.7%
Four weeks	14	14	16	15	14.75	64.7%
Control (four weeks)	42	40	45	41	42.0	00.0%

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NOTICES

JOHN LEWIS SHELDON. The death of John Lewis Sheldon, professor of bacteriology and later of botany at West Virginia University, 1903-1919, occurred at Morgantown, W. Virginia, on Jan. 15, 1947, in his 82nd year. Dr. Sheldon took his doctorate at the University of Nebraska under the guidance of Dr. Charles Bessey, that most eminent of American botanical teachers, and was one of the group of Bessey's early students who have had a marked influence on American botany.

During his active years at West Virginia Sheldon carried out a series of phytopathological studies, giving particular attention to the taxonomy and life histories of the several fungi concerned. His published papers include reports on fungi associated with apple and guava diseases, on various anthracnose fungi, and on a number of rusts. Among fungi named by him were the ubiquitous *Fusarium*

moniliforme and *Illosporium malifoliorum*, the latter associated with an apple leaf spot.

Following his withdrawal from the University he continued his interest in the botany of his adopted State, studying especially mosses, fungi, and lichens. His studies of the latter culminated in an extensive paper on the lichens of West Virginia (*Castanea* 4: 75-136. 1939), his last published paper. His extensive botanical collections presumably will be ultimately incorporated into the herbarium of West Virginia University.

More extended accounts of the life and work of Professor Sheldon appeared in *Castanea* (4: 69. 1939) and in *Science* (105: 541. 1947). The first of these includes a portrait and a bibliography.—JOHN A. STEVENSON.

PAUL MARSHALL REA. News has been received that Paul Marshall Rea, former director of the Santa Barbara Museum of Natural History, died suddenly at his home, 436 E. Padre Street, Santa Barbara, California, January 15, 1948. As a member of the Mycological Society he was active in the study of the fleshy fungi of the region around Santa Barbara, and he built up an herbarium of more than 1,600 specimens. He published on a number of his discoveries, and at the time of his death was occupied with a study of *Lysurus*. His greatest fame, however, was in museum circles where he was nationally known. He was a former president of the American Association of Natural History Museums, and author of several books on this type of museum and its relation to the community.

He was born in Potuit, Massachusetts, February 13, 1878, was a graduate of Williams College and did graduate work at Columbia University. He taught at the University of South Carolina before going to Cleveland as the first director of the Cleveland Museum of Natural History. In 1931 he resigned from the Cleveland Museum and went to Santa Barbara and became the director of the natural history museum there. He retired in 1941. He is survived by Mrs. Marion Goddard Rea; a daughter, Dorothy Helen Rea of Berkeley, California; and a son, John Morse Rea of Garden City, L. I., and three grandchildren.—ALEXANDER H. SMITH.

HENRY C. BEARDSLEE. News reached me a short time ago of the death of Mr. Beardslee, one of our well known members and for many years a critical student of the fleshy fungi. A more detailed account of his life and work will appear in a forthcoming issue of *Mycologia*.—ALEXANDER H. SMITH.

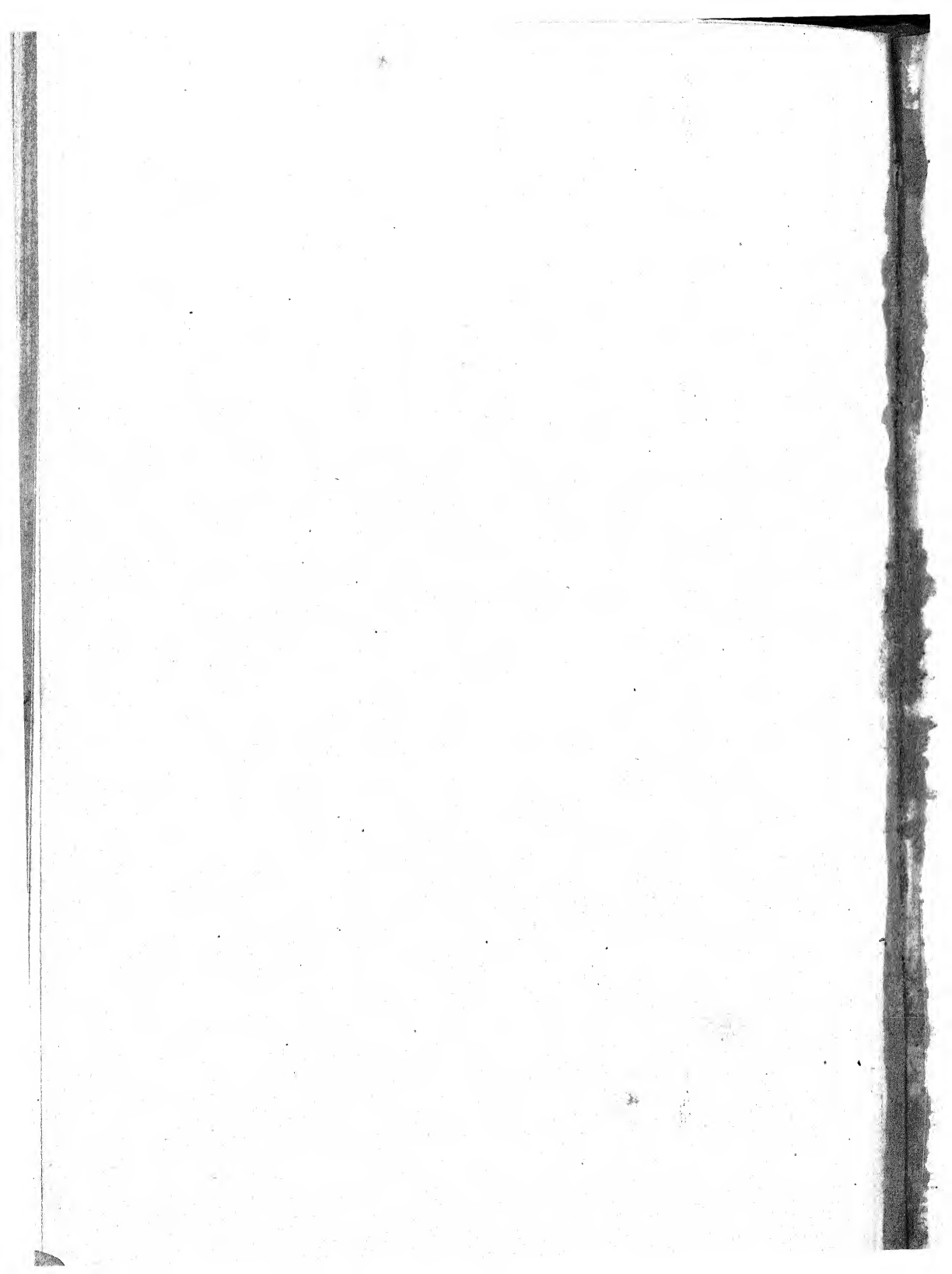
WILLIAM H. LONG. The death of William H. Long, retired forest pathologist of the Bureau of Plant Industry, and for some years a member of the Mycological Society, is noted. Dr. Long passed away at his home in Albuquerque, New Mexico, where he had lived since retirement in 1937, on December 10, 1947. A more extensive account of his life and work will be presented later.—JOHN A. STEVENSON.

CORRECTIONS: In the article by Roberts in the March-April issue the figures were interchanged. Fig. 1 should read *N. profusa* and Fig. 2 *N. ramosa*.—A. H. S.

In the recently published directory of the Society H. R. Rosen's address was erroneously given as Rahway, N. J. It is Univ. of Arkansas, Fayetteville, Ark.—A. H. S.

WANTED—*Mycologia*, vols. 31, 32, 36, 37

The stock of volumes 31, 32, 36 and 37 of *MYCOLOGIA* is running low, and of certain numbers is exhausted. To fill future orders for back sets it is desirable to build up the supply. Until enough are again on hand, these volumes or the needed parts will be purchased at the rate of \$5.00 a volume. Those who do not need and are willing to sell these volumes will assist the journal by reporting that fact to the Managing Editor, *Mycologia*, New York Botanical Garden, New York 58, N. Y.—DONALD P. ROGERS.



MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XL

JULY-AUGUST, 1948

No. 4

AN UNDESCRIBED SPECIES OF PAPULOS- SPORA PARASITIC ON RHIZOCTONIA SOLANI KUHN

JOHN R. WARREN

(WITH 3 FIGURES)

In an investigation to determine the cause of the black-root disease of sugar beets in Ohio, 690 isolations were made from diseased seedlings. Seventy or 7.6 per cent of the total number of isolations yielded *Rhizoctonia solani* Kuhn. As expected, the fragments of beet tissue from which *R. solani* was isolated frequently yielded cultures of other species of soil fungi. One of these, which appeared with greater frequency than any other species, was an organism subsequently recognized as an undescribed species of *Papulospora*. This fungus was isolated thirty times and it constituted 4.3 per cent of the total number of isolates. Although usually isolated from seedlings from which *R. solani* was also isolated, occasionally this *Papulospora* was the only fungus obtained from apparently diseased seedlings.

In the investigation of sugar beet black-root a series of tests were made to determine the pathogenicity of representatives of all species of fungi isolated. These tests included six isolates of *R. solani* and six isolates of the *Papulospora*. Although they were of varying degrees of virulence, all of the tested isolates of *R. solani* caused symptoms of the black-root disease. Two of the six isolates were extremely virulent and prevented the survival of any seedling beets growing in the infected soil. The number of seed-

lings surviving ranged from twenty-five per cent to 100 per cent less than the number of seedlings in pots of soil free of the organism.

The pathogenicity tests made with the isolates of *Papulospora* were negative in that a stand of seedlings similar to that in the control pots was obtained and none of the symptoms characteristic of black-root were developed. There was, however, a marked stunting, or reduction in the rate of growth, of the seedlings growing in soil to which this organism had been added as compared with those growing in soil free of the fungus. When the stunted beets were removed from the soil and examined microscopically, there was no evidence that they had been invaded by the *Papulospora*.

Although the *R. solani* isolates were extremely virulent in pot tests, it is unusual for a field planting of sugar beets to be completely destroyed by black-root caused by this organism. This seemed to indicate either a limited occurrence of the fungus in the soil of the beet-producing area or an antagonistic condition between *R. solani* and other soil fungi. To determine if the latter possibility was involved, several species of fungi were tested to determine if a reaction occurred between them and *R. solani*. Among the fungi so tested was the species of *Papulospora* which had occurred so frequently in culture with *R. solani*.

METHODS

The pathogenicity tests of the two species of fungi were made by mixing ten day old cultures of the organisms with soil taken from sugar beet fields and steam sterilized. The cultures serving as inoculum were growing on a corn meal-sand medium. After the soil was mixed with the inoculum, six inch, unglazed pots were filled with the mixture and a moderate amount of water was added to the surface. Three days later the pots of soil were seeded with sheared seed of a type used in the commercial beet fields of Ohio. Twenty-five seed pieces were placed in each pot of soil and covered to a depth of one quarter inch. Control pots containing a mixture of sterilized soil and the corn meal-sand medium were prepared and maintained under the same conditions as the test pots.

In preliminary tests for antagonism between the fungi the organisms being tested were plated with *R. solani* on two per cent agar media in Petri dishes. The media used included potato-dextrose, prune, carrot, lima bean, and corn meal agars.

To determine the microscopic relationship between the two fungi when they grew together, slide cultures were made. For this purpose two large loopfuls of melted potato-dextrose agar or corn meal agar were placed in the center of flamed, vaseline-ringed cover slips. After the medium had cooled, hyphal transplants of the organisms were made from actively growing cultures to the opposite sides of the drop of medium and the cover slips were inverted over flamed, hollow ground slides.

To determine the behavior of the two fungi in the soil, microscope slides were buried in pots of previously sterilized soil which had been mixed with cultures of the two organisms growing separately on a corn meal-sand medium. Ten to fifteen days later the slides were carefully freed from the soil, air dried, and stained by immersion in carbol fuchsin maintained at 100 degrees centigrade in a water bath.

The effect of the presence of the *Papulospora* in reducing the amount of sugar beet root rot caused by *R. solani* was evaluated by a series of pot tests. In these tests ten day old cultures of each of the fungi growing separately on corn meal-sand medium were mixed with steam sterilized soil. Pots of soil containing *R. solani* alone were also established as were control pots. Twenty-five seed pieces were planted in each six inch pot of soil-culture mixture.

OBSERVATIONS AND EXPERIMENTAL RESULTS

When this species of *Papulospora* grew on the same plate of medium with *R. solani*, conspicuous results were obtained. Along the line of juncture of the colonies there developed, as shown (FIG. 1), a band of mycelial growth denser in character and whiter in color than that of other parts of the culture. With increasing age this band continued to widen until the area over which the *R. solani* had grown was covered with the dense, white growth.

A microscopic examination made in the region of the denser growth revealed that the mycelium of the *Papulospora* had over-

grown that of the *R. solani* and that the hyphae of the latter had become entwined by those of the *Papulospora* as shown (FIG. 2). Uniform coils were formed, the number on any one hypha increasing with the age of the culture. In cultures eight to ten days old complete ensheathment of the *R. solani* hyphae had usually resulted as, shown (FIG. 3).

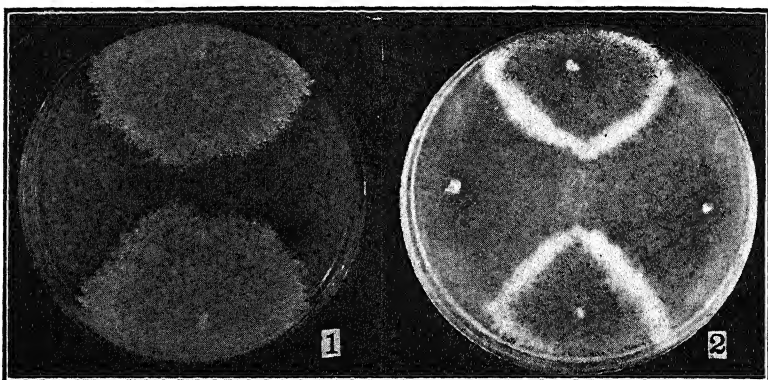


FIG. 1. Petri dish cultures of *Rhizoctonia solani* and *Papulospora stoveri*.

The cover slip-slide cultures showed that there was no apparent physiological attraction between the hyphae of the two fungi when they were separated from each other. After the hyphae, growing from opposite sides of the culture, had come into contact with each other the growth rate of the parasite was increased and the subsequent extension of the *Papulospora* paralleled the older hyphae of *R. solani*. Usually after the hyphae had been in contact for a distance of twenty to thirty microns lateral branches developed on the *Papulospora* hyphae. These grew rapidly, coiling around the hyphae of *R. solani*. In some instances less than ten minutes were required for the production of a single coil.

When a lateral branch of the *Papulospora* first began to form, the protoplasm of the *R. solani* hyphae separated into two parts each of which retracted from that part of the hypha nearest the developing branch. By the time several encircling branches had developed, the protoplasm of the host had disintegrated and only the walls of the hypha remained. Occasionally, instead of lateral

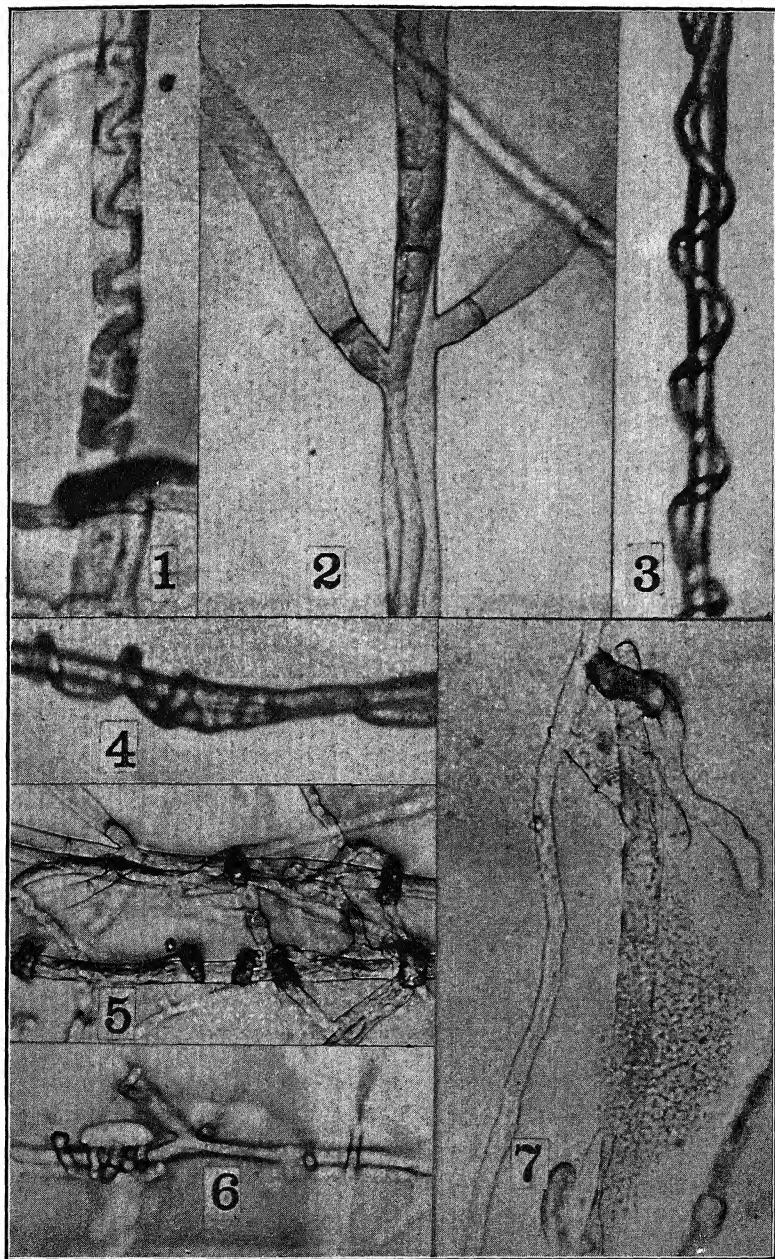


FIG. 2. Photomicrographs of *Rhizoctonia solani* parasitized by *Papulospora stoveri*.

branches developing, the *R. solani* hyphae were entwined by the *Papulospora* hyphae as the latter grew in length.

Although the interaction of the two fungi was predominantly as just described, it was not unusual to find numerous instances of the penetration of the *Papulospora* hyphae into those of *R. solani*. By removal of lengths of internally parasitized hyphae and examination with the aid of a micromanipulator it became apparent that the hyphae of the *Papulospora* had penetrated and were growing within those of *R. solani* as shown in figure 2.

TABLE I
THE EFFECTIVENESS OF A SPECIES OF *Papulospora* IN REDUCING THE
AMOUNT OF SUGAR BEET BLACK ROOT CAUSED
BY *Rhizoctonia solani* KUHN

<i>Rhizoctonia solani</i> Isolate Number	Number of Sugar Beet Seedlings Surviving in Soil Containing only <i>Rhizoctonia solani</i>			Number of Sugar Beet Seedlings Surviving in Soil Containing <i>Rhizoctonia solani</i> plus <i>Papulospora stoveri</i>		
	Series A ¹	Series B ¹	Series C ²	Series A ¹	Series B ¹	Series C ²
1-a	0	0	6.5	.5	0	11.0
A1-20	3.0	0	3.0	3.5	1.0	17.0
64-4	0	1	2.5	3.5	3.2	2.7
H-10	7.5	9.5	11.2	14.0	8.5	20.4

A. Seeds planted four days after cultures and soil were mixed.

B. Seeds planted twenty-four days after cultures and soil were mixed.

C. Seeds planted fifty-five days after cultures and soil were mixed.

¹ Average of seedlings in four pots.

² Average of seedlings in eight pots.

This species of *Papulospora* parasitized *R. solani* when cultivated on a wide variety of media; and by use of buried slides, as previously described, it was found that parasitism also occurred in the soil. The parasitism in the soil occurred in the same manner as in Petri dish or cover slip-slide cultures.

It remained to be determined by a series of pot tests whether *Papulospora* in the soil could effect a reduction in the amount of black-root of sugar beet seedlings caused by *R. solani*. Hence, ten day old cultures of *R. solani* were mixed with soil in the amount of one 400 ml. flask of culture to two six inch pots of soil. Eight pots of soil were prepared for each of the four isolates. One-half of these (four pots of soil-culture mixture for each isolate) were then mixed with ten day old cultures of the *Papulospora*. Four

days later, two pots from the four for each isolate of *R. solani* and two pots from the four for each isolate of *R. solani* plus the *Papulospora* were planted with twenty-five beet seed pieces to each pot. The results of this planting are shown under the heading, Series A of Table 1. The remaining pots of soil were watered at frequent intervals and planted with beet seed twenty-four days after the cultures and the soil were mixed. The results of this test are headed Series B in Table 1. Fifty-five days after cultures and soil were mixed, all of the pots were replanted. These results are shown in Series C of Table 1. In each of these tests the seedlings were examined for symptoms of the disease ten days after planting.

From the results of this test it appears that this species of *Papulospora* is effective in reducing the incidence of disease caused by some but not all strains of *R. solani*, and that the efficiency increases as the interaction time is increased.

DISCUSSION

In this study the observations made of Petri dish cultures, cover slip-slide cultures, and of slides buried in soil containing *R. solani* and the *Papulospora* indicate an actual parasitism of *R. solani* by the *Papulospora*. This type of parasitism is in many respects similar to that described by Weindling (10) of the parasitism of *Trichoderma lignorum* (Tode) Harz. on *R. solani*.

There are numerous publications dealing with antagonism among microorganisms but those concerned with the actual parasitism of one fungus by another one are comparatively meager. Types of parasitism have been reported by Zopf (12, 548-551) and by Reinhardt (7) and detailed studies have been made by Blochwitz (2) and by Ayers (1) on the parasitism of some of the Mucorales.

The results of the cultural studies indicate that although the *Papulospora* grows well alone in culture it does obtain some substances from *R. solani* when parasitism occurs. Indirect evidence of this is the plasmolysis and subsequent disintegration of the *R. solani* protoplasm. Somewhat more direct evidence is the close paralleling of *R. solani* hyphae by those of the parasite, the increased growth rate, and increased bulbil formation of the *Papulospora* after parasitism has occurred.

The evidence obtained from pot tests in this study with *R. solani* and *Papulospora* indicates that given a sufficient interaction time, the parasitism of *R. solani* on sugar beet seedlings is significantly reduced. These tests also indicate that there are some strains of *R. solani* which are little affected by the *Papulospora*.

This possibility of suppressing the growth of phytopathogenic fungi by microbiological activities has been suggested by several experimenters. Sanford and Broadfoot (9) have shown that root rot of cereals caused by *Ophiobolus graminis* Sacc. can be controlled by the activities of various soil inhabiting microorganisms. Sanford (8) and Millard and Taylor (6) explain the reduction of potato scab, when a green rye crop is plowed down, as being due to the increased activity of other soil microorganisms. Greaney and Machacek (4) found the pathogenicity of *Helminthosporium sativum* Pamm., King and Bakke, a root rotting agent of cereals, was determined in part by the activity of *Cephalothecium roseum* Cda. However, in each of these studies the effects observed are apparently due to the production of diffusible substances rather than to parasitism.

King and his associates (5) have experimented with the control of *Phymatotrichum omnivorum* (Shear) Duggar, the cause of a root rot of cotton, by altering the soil microflora with organic amendments. Brown (3) has indicated that root rot of watermelons caused by *P. omnivorum* may be reduced by the presence of *Trichoderma* spp. in the soil. Weindling and Fawcett (11) have suggested that the soil acidification which caused a reduction in the damping off of citrus seedlings might be due primarily to the increased parasitic, antagonistic, and competitive effects of other soil fungi.

The results obtained by these investigators indicate the possibility that future studies will demonstrate that the soil population of *Papulospora* may be increased and the interaction time required for the destruction of *R. solani* decreased by suitable soil amendments.

Since this species of *Papulospora* forms bulbils copiously in culture, it was sent to Dr. J. W. Hotson for examination because of his extensive knowledge of bulbiferous fungi. Dr. Hotson has informed the writer that this species has not previously been de-

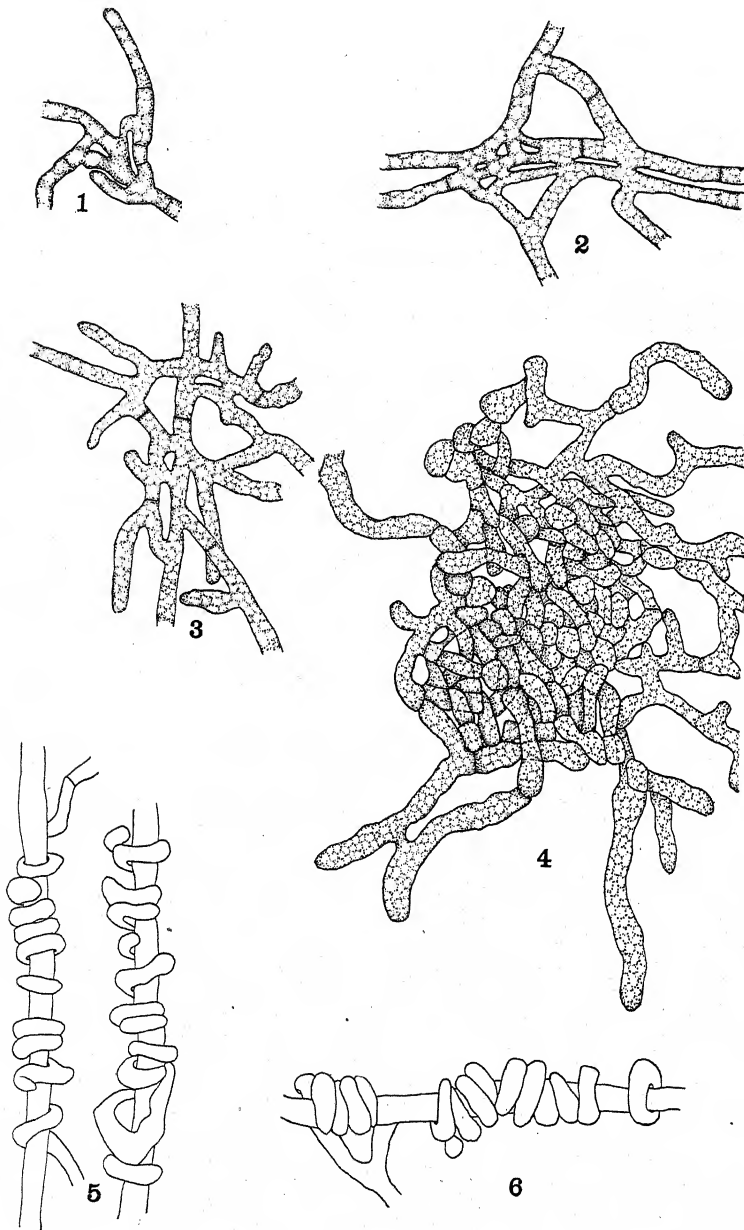


FIG. 3. Bulbil formation by *Papulospora stoveri*. 5 and 6, *Rhizoctonia solani* parasitized by *Papulospora stoveri*.

scribed. The description is as follows and the manner of bulbil formation is shown in figure 3.

Papulospora stoveri sp. nov.

Mycelium pallide luteum, procumbens, profusum; superficiei hyphis $5.7\ \mu$ diametro, submersis hyphis $6.7\ \mu$ diametro; inaequaliter septatis, ramosis, cum magnis globulis refractivis. Bulbilli colore maturi, rufo-brunnei; $630 \times 488\ \mu$, variables ($480\text{--}640 \times 384\text{--}488\ \mu$); hyphae primordiales cellularum multarum intercalarum. Conidia absunt.

Hab. in argillosa terra et in radicibus *Betae vulgaris* novellis prope Fremont, Ohio. U. S. A.

Mycelium light buff, procumbent, profuse, growing in and on the medium. Surface hyphae $5.7\ \mu$ in diameter, submerged hyphae $6.7\ \mu$ in diameter, irregularly septate, much branched, with large refractive globules. Bulbils burnt sienna when young changing to reddish brown at maturity; size ranging from $480 \times 384\ \mu$ to $640 \times 544\ \mu$. Average size $630 \times 488\ \mu$; primordium many intercalary cells. No conidia are produced.

Habitat. From clay soil and from the roots of sugar beet seedlings near Fremont, Ohio. U. S. A.

SUMMARY

1. A previously undescribed species of *Papulospora*, isolated from soil, is described.

2. This species has been found to be parasitic on *R. solani* by both an internal and an external type of parasitism.

3. The parasitism of the *Papulospora* on *R. solani* has been found, in pot tests, to be effective in reducing the amount of sugar beet black-root caused by *R. solani*.

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DESCRIPTION OF FIGURES

FIG. 1. Petri dish cultures of *Rhizoctonia solani* and *Papulospora stoveri*. 1. *R. solani* alone, 2. *R. solani* and *P. stoveri*.

FIG. 2. *Papulospora stoveri* parasitizing *Rhizoctonia solani*. 1-3, $\times 600$; 4-6, $\times 300$; 7, $\times 450$.

FIG. 3. 1-4, Camera lucida drawings of bulbil formation by *Papulospora stoveri*. $\times 300$. 5 and 6, Camera lucida drawings of *Rhizoctonia solani* hyphae parasitized by *Papulospora stoveri*. $\times 300$.

ENTOMOGENOUS FUNGI¹

E. B. MAINS

(WITH 4 FIGURES)

CORDYCEPS PUIGGARII Speg.

In 1882, Spegazzini (16) under the name "*Cordyceps* (*Torrubia*) *sphaecophila* (Kl.) Berk. and Curt."² published a description of a fungus collected by J. Puiggari on *Polybia fasciata* in Brazil. Later, in 1889, he (17) decided that it was a new species for which he proposed the name *Cordyceps Puiggarii*. In 1919, Spegazzini (18) again employed the name *C. Puiggarii* for a fungus which he designated as a new species. It was based on two collections of J. Puiggari (Nos. 141 and 154) on a beetle, *Lystronchus* sp. It is evident from the descriptions that two different species are involved which therefore cannot bear the same name. Petch (13) has suggested that the collection on *Polybia* may be *C. sphaecocephala* and those on *Lystronchus*, *C. curculionum*. The orientations of the perithecia and the sizes of the asci as given by Spegazzini raise questions concerning the identities of the collections. Through the kindness of Juan C. Lindquist, it has been possible to study the specimens in the Spegazzini Herbarium of the Museo de la Plata.

The collection on *Polybia fasciata* (*Herb. Speg. 1771*) is labeled "*Cordyceps Puiggarii* Speg. Typus." On an inner packet "*C. sphaecocephala* (Kl.) B. & Br." and "*C. Humberti* Rob." are written along with some notes and sketches. The collection consists of several broken clavae arising from the thorax of the insect

¹ Paper from the Department of Botany and the Herbarium of the University of Michigan. The cost of two extra plates has been paid by the Herbarium of the University of Michigan.

² The name *Cordyceps sphaecophila* Berk. & Curt. evidently is an error. Berkeley and Curtis (2) give *Torrubia sphaecophila* Tul. as a synonym. However, Tulasne (20) published it as *Torrubia sphaecocephala* and referred to *Sphaeria sphaecocephala* Klotzsch.

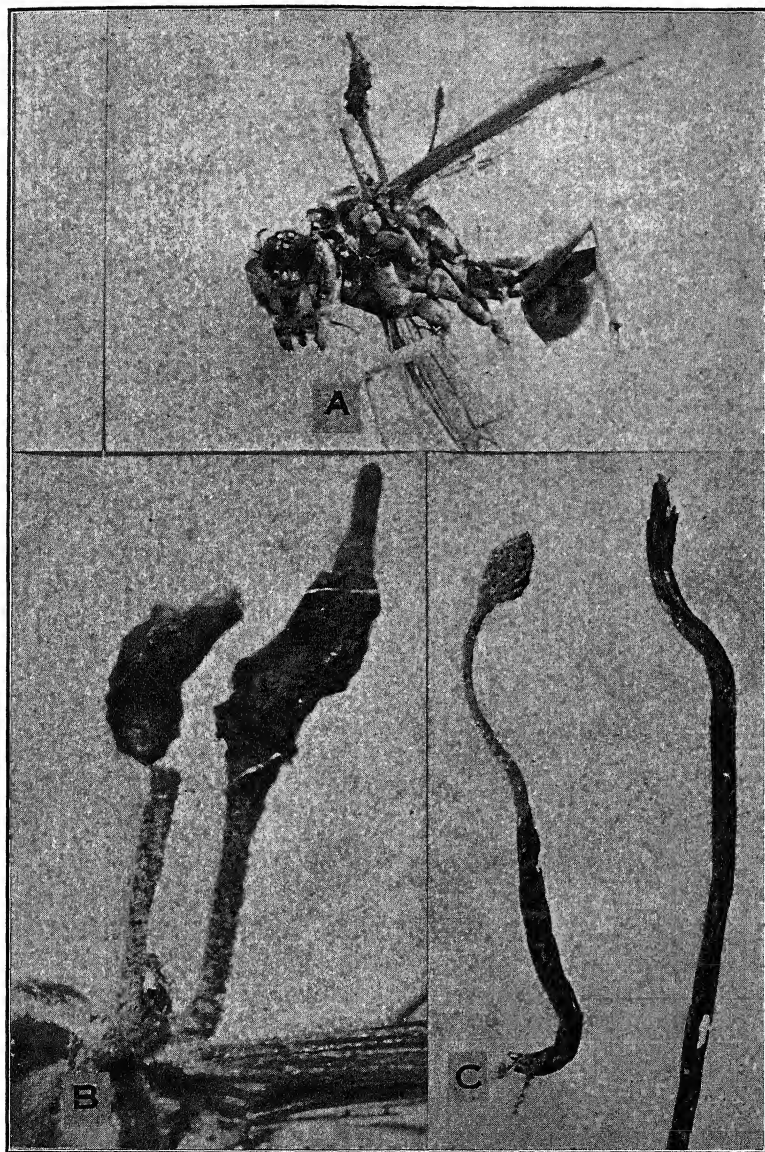


FIG. 1. *Cordyceps Puiggarii* Speg.

(FIG. 1, A & B). The fertile portions are broken off from all except two, and one is loose in the packet. The following data have been obtained from a study of the collection.

Clavae fusoid, 3–5 mm. long, the fertile portion 1–2 mm. long, 0.3–0.7 mm. thick, narrowed above into a sterile acuminate apex up to 1 mm. long, narrowed below into a stipe approximately 2–3 mm. long, 0.2 mm. thick, ochraceous-buff, the fertile portion olive due to the perithecia, the stipes noticeably puberulent specially below; perithecia embedded, prominent, producing an irregular surface, broadly ovoid to globoid, $320\text{--}420 \times 300\text{--}320 \mu$, at right angles to the surface of the clava; asci fusoid-cylindric, narrowing to apex and base, $132\text{--}180 \times 9\text{--}10 \mu$, the wall slightly thickened at the apex (2μ); ascospores fusoid-cylindric, $90\text{--}110 \times 2\text{--}2.5 \mu$ overlapping in the ascus, multiseptate, not or tardily breaking into fragments. On *Polybia fasciata*, Apiaty, Brazil, V. 1881, Puiggari.

This is not *C. sphecocephala* which has oblique, much larger perithecia, cylindric asci up to 660μ long and ascospores which early break into one-celled fragments. On account of similar differences it cannot be placed in synonymy with *C. oxycephala* Penz. & Sacc. as has been proposed by Kobayasi (5). From the notation on the inner packet, Spegazzini apparently considered the possibility that the collection might be *C. Humberti*. In general appearance it resembles *C. Humberti* as illustrated by Saussure (15). It also does not differ greatly in microscopic details from *C. Humberti* as described by Petch (13). Saussure did not figure sterile apices for the clavae. Petch has suggested that they were probably broken off. The puberulence of the stipes which is very noticeable in the type specimen of *C. Puiggarii* has not been described for *C. Humberti*. Apparently the two species are very closely related, but it seems best to recognize them as distinct from each other.

Of the specimens on the beetle, *Lystronchus* sp., No. 141 of Puiggari (*Herb. Speg. 1773*) consists of fragments of a beetle and a short portion of a brown stipe. Specimen No. 154 of Puiggari (*Herb. Speg. 1772*) consists of three fragments of stipes and two heads (FIG. 1, C). The clavae evidently were bicolored. The fragments of the stipes are brownish-black except for short portions of two which are a light yellowish-brown. The heads are

concolorous with the yellowish-brown portions of the stipes. The heads are fusoid-ovoid. The perithecia are narrowly ovoid, $780-960 \times 216-300 \mu$, obliquely embedded and overlapping upward. The asci are narrowly cylindric, up to 480μ long and $6-8 \mu$ wide. Only immature ascospores were found. These collections are *C. curculionum*.

CORDYCEPS SUBSESSILIS Petch

Cordyceps subsessilis was described by Petch (14) from collections made by Roland Thaxter in North Carolina and Tennessee. The development of the fungus is somewhat unusual for a *Cordyceps* (FIG. 2 A, Type). Petch states "it would appear that the fungus produces perithecia as soon as its mycelium reaches the surface of the wood, the apparent stalk being merely the strand of mycelium in the wood, probably in the insect bore-hole."

This species appears to have an aberrant development of the stipe. Two collections made by A. H. Smith several years ago show a similar development. One of these (14573) arises from a white mycelial mass with fragments of an insect probably a beetle (FIG. 2, C). A stalk-like portion, which was 5 mm. long and 4 mm. wide when collected, is crowned by a poorly defined head consisting of vertical perithecia partly embedded in a white stroma. In the other collection (7724) the two heads terminate white stalk-like strands 5 mm. long and 0.5-1.2 mm. thick (FIG. 2, B). The heads form slight terminal enlargements 0.8-1.8 mm. wide. Most of the perithecia are embedded except for their apices. A few are covered only in the basal portion apparently due to the shrinkage of the stroma. In both of these collections only the groups of perithecia showed above the rotten wood. The heads are connected to the buried insects by stalk-like strands. The asci are very long and narrow, measuring $450-600 \times 3.5-4 \mu$. The embedded perithecia and the long asci do not agree with the description given by Petch who describes the perithecia of *C. subsessilis* as free and glabrous and the asci as 240μ long. Through the kindness of Dr. Rolf Singer it has been possible to study the specimens from the Farlow Herbarium cited by Petch. Although most of the perithecia of the type specimen appear to be free, in a

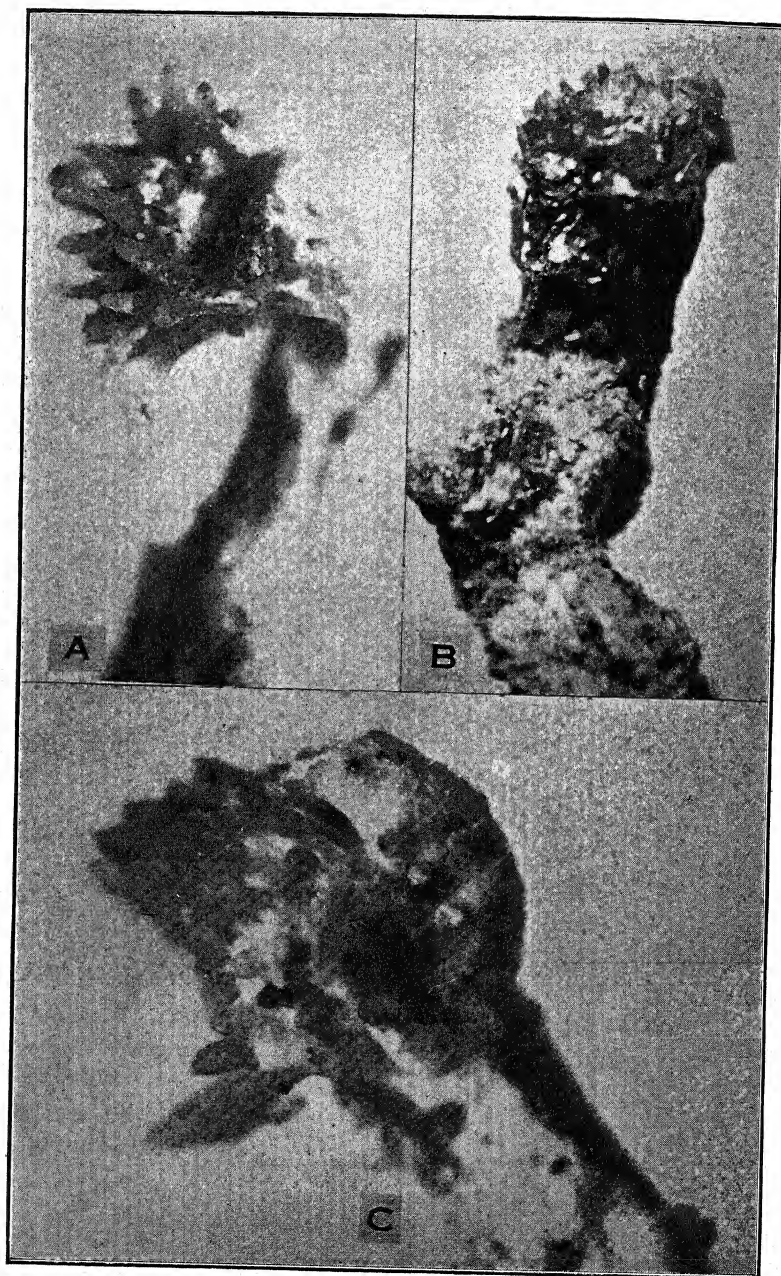


FIG. 2. *Cordyceps subsessilis* Petch.

small group they are covered except for their apices by a soft white stroma (FIG. 2, A). It would appear that typically they are covered at first and later become free by the shrinkage and disappearance of the stroma. The asci measure $430-540 \times 4 \mu$. The following description is offered.

Clavae poorly developed, the stipes entirely included in the rotten wood of the substrata, developing irregularly, apparently reaching the surface through borer-holes from buried coleopterous larvae, brownish white with brown patches, 4.5-6 mm. long, 0.5-4 mm. thick, slightly enlarging on reaching the surface to form irregular heads 0.8-5 mm. wide, the heads at first white becoming light brown on account of the exposed perithecia; perithecia at first embedded in a soft stroma, later becoming mostly free and appearing superficial due to the shrinkage and disintegration of the stroma, light brown, conoid to narrowly ovoid, $900-1080 \times 324-444 \mu$; asci narrowly cylindric, $430-600 \times 3-4 \mu$, the ascospores filiform, breaking into part spores $3-6 \times 0.5-1 \mu$.

On coleopterous larvae buried in rotten wood. Burbank, Tennessee. Aug. 1896, R. Thaxter (*Farlow Herb. No. 6145, type*); Cranberry, N. C., 1887, R. Thaxter (*Farlow Herb. No. 6135*);³ Drahner Road, Oakland County, Mich., Sept. 24, 1937, A. H. Smith (7724); Elwha River, Wash., June 22, 1939, A. H. Smith (14573).

In discussing the species Petch (14) refers to the stipes as a pseudostalk. He expresses some doubt whether the species should be referred to *Cordyceps* or to *Torrubiella*. In *Torrubiella* the perithecia develop in a stroma covering the host. It therefore would seem best to consider this a species of *Cordyceps* with an aberrant stipe.

CORDYCEPS PELTATA Wakefield

In 1916 Miss Wakefield (21) described a very interesting and unusual entomogenous fungus on *Cryptorhynchus* from the West Indies and named it *Cordyceps peltata*. Only the peltate heads were developed above the substratum. The stipes are described as short, entirely immersed in the substratum. The clavate asci contain fusiform multiseptate ascospores which, unlike ascospores

³ Specimen consists of a few fragments and a number of loose perithecia.

of this type in other species of *Cordyceps*, separate at maturity into two halves. As far as I have been able to ascertain, this species has not been reported again. Several years ago it was noted that a specimen is deposited in the Farlow Herbarium from Barbados Island. Through the kindness of Dr. Rolf Singer it has been possible to study the specimen and prepare the following description.

Fertile portions of the fructifications peltate, closely appressed to the substratum, 2–3 mm. across, 0.7–1.0 mm. thick, coalescing to form an irregular series 10 mm. long, each apparently developing an umbo at the center, vinaceous-buff when immature becoming chestnut with vinaceous-buff margins at maturity, punctate on the upper surface from the slightly projecting ostioles of the perithecia; stipes short, up to 2 mm. long, completely embedded in the substratum, irregular, up to 1.5 mm. wide above, narrowing below, ochraceous-buff, arising from a brownish horizontal rhizomorph-like strand which develops from a dense white mycelial covering of the insect; perithecia vertical, opening on the upper surface of the head, ovoid, $430\text{--}480 \times 168\text{--}204 \mu$; asci fusoid-clavate, attenuated below, narrowed above, $176\text{--}192 \times 9\text{--}11 \mu$; the wall not noticeably thickened at the apex; the ascospores slightly overlapping in the upper portion of the ascus, fusiform, $72\text{--}84 \times 3\text{--}4 \mu$, multiseptate, at maturity breaking into half-spores (FIG. 3, A & B).

On *Cryptorhynchus corticalis* Boh., Barbados, det. R. Thaxter (Farlow Herb. 4046).

The plant infested by the larva is not identified. Miss Wakefield states that the larva of her collection infested cultivated *Cordia*. The larva of the Barbados specimen occurs in a burrow and is surrounded by a dense white mycelial covering. From this a rhizomorph-like strand developed in the wood and produced progressively a number of fructifications at short intervals as indicated by variations in maturity (FIG. 3, A, from left to right). The crowding of the fructifications resulted in a coalescing of the peltate heads in an irregular line. Each head apparently produced a conical umbo in the center. All except the one on the youngest head to the extreme right are broken off, leaving only scars.

Lloyd (7) has commented on the unusual characters of this species and has questioned its inclusion in *Cordyceps*. He has suggested a close relationship to *Hypomyces* and *Hypocrea* and states

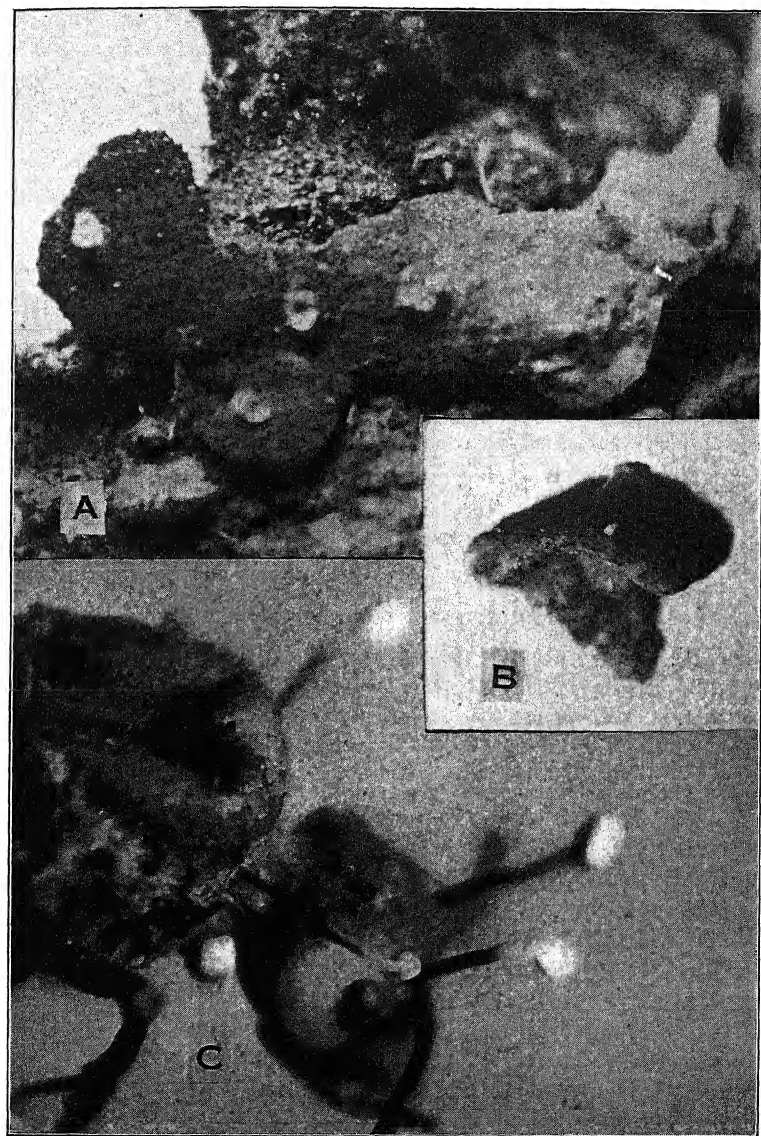


FIG. 3. *Cordyceps peltata* Wakefield.

that it "would be *Clintonella peltata* on spore character alone." Although the almost sessile heads might at first glance be mistaken for stromata of a *Hypocrea*, as Miss Wakefield has pointed out, this is only a superficial resemblance. Short, poorly developed stipes are formed. The long, narrowly fusiform, multiseptate ascospores do not resemble spore types of *Hypocrea*. A number of species of *Cordyceps* have similar spores and Petch (10) has separated these as a genus *Ophiocordyceps* including *C. peltata* as *Ophiocordyceps peltata* (Wakefield) Petch. The separation of the ascospores into half-spores is not found in other species of the group. The writer (8) has questioned the generic separation of the species of *Ophiocordyceps*, and Kobayasi (5) has recognized *Ophiocordyceps* as a subgenus of *Cordyceps*. *Cordyceps peltata* is a unique species which with our present knowledge of the genus cannot be placed in close relationship with other species. It appears to occupy an intermediate position between *Ophiocordyceps* and *Eucordyceps* types.

Stilbum burmense sp. nov.

Synnemata 1-3 mm. longa, capitata, capitulis globosis vel late obovoideis, 720-984 μ latis, pallide cremeis, stipitibus cylindraxis, 180-300 μ crassis, dorsum fuscoatris, sursum concoloribus capitulis; phialides densissime dispositae, anguste clavatae vel cylindraxis, 22-28 \times 2-2.5 μ , apice unicum conidium gignentes; conidia hyalina, anguste obovoidea, 5-8 \times 2 μ . Ex formica, Myitkyina, Burma, W. L. Jellison, 1945.*

Synnemata arising from various parts of the body and appendages of the host, 1-3 mm. long, capitate, the heads globoid to broadly obovoid, 720-984 μ broad, light cream color, the stipes cylindric, 180-300 μ thick, brownish black below, concolorous with the heads in the upper portions, consisting of parallel, longitudinal hyphae, the inner colorless, 2.5 μ thick, thin-walled and sparsely septate, the outer in the lower portion of the stipe brownish, 4 μ thick and with septa 8-30 μ apart; phialides forming a very dense peripheral layer of the head, at right angles to the surface, narrowly clavate to cylindric, 22-28 \times 2-2.5 μ ; conidia hyaline, covered with a mucus, adhering to form a covering over the head upon drying, narrowly obovoid, 5-8 \times 2 μ , smooth, produced singly at the apices of the phialides.

On a flying ant, Myitkyina, Burma, W. L. Jellison, 1945.

This interesting specimen was received from Edward A. Steinhäus.

The cream colored portions of the bicolored synnemata were probably some shade of red when fresh. *Stilbum formicarum* Cooke and Massee which also has bicolored synnemata was described (3) on an ant from Australia. It was described as having black stipes and obovate, roseous heads. Petch (12) has studied the specimen in the Kew Herbarium and states that the synnemata are linear with the apices acute, obtuse or slightly inflated into a head. He describes the conidia as broadly clavate or obovate, $6-9 \times 2.5-4 \mu$, and the phialides as cylindric or clavate, $15-18 \times 3 \mu$, apparently forming a palisade layer over the synnemata. Petch reaches the conclusion that *S. formicarum* is a *Hymenostilbe* and is a synonym of *H. melanopoda* Petch. The smaller spores, the definitely capitate synnemata and the limitation of the phialides to the head distinguish *Stilbum burmense*.

The bicolored synnemata of *S. burmense* strongly suggest that it may be the conidial stage of one of the species of *Cordyceps* of ants having bicolored clavae. *Cordyceps australis* (Speg.) Sacc., *C. bicephala* Berk., *C. necator* Pat. and Har., *C. proliferans* P. Henn. and *C. Huberiana* P. Henn. have been described as producing bicolored clavae on ants. Petch (11) states that *Hymenostilbe melanopoda*⁴ is the conidial stage of *Cordyceps bicephala*. This conclusion is apparently based on the following statement by Spegazzini (17) concerning *Isaria melanopus*, "Species statum conidicum Cordicipitis australis Speg. facillime sistens." Spegazzini gives the hosts of *I. melanopus* as Coleoptera and it would seem very doubtful that it would be the conidial stage of a parasite of ants. A connection with *Cordyceps curculionum*, a bicolored species infecting beetles, would appear to be a more likely possibility. For a similar reason it is doubtful that *Stilbum formicarum* is synonymous with *Hymenostilbe melanopus* (*H. melanopoda*). Since the combination *Hymenostilbe formicarum* is pre-empted (Petch, 12) the name ***Hymenostilbe australiensis*** nom.

⁴ Petch (11) in transferring *Isaria melanopus* Speg. to *Hymenostilbe* changed the specific name, proposing the combination *Hymenostilbe melanopoda*. This is not justified and the combination should be *Hymenostilbe melanopus*.

nov. (*Stilbum formicarum* Cooke and Massee) is proposed. Both *Hymenostilbe australiensis* and *Stilbum burmense* are probably conidial stages of bicolored species of *Cordyceps* infecting ants. Until they are found associated with their ascigerous stages their specific connection will remain uncertain.

Mycologists are not in agreement concerning the application of the generic name *Stilbum*. Tode (19) proposed the name in 1790 for a genus, describing six species, the first being *Stilbum vulgare*. Fries (4) in 1832 in his treatment of *Stilbum* in his *Systema Mycologicum* included twenty-two species. The first species described is *Stilbum hirsutum*. *S. vulgare* is the nineteenth. Fries placed the genus in his third class, Hyphomycetes. For many years *Stilbum* has been used for a genus of the Hyphomycetes and more than 100 species have been described (140 included by Saccardo according to Ainsworth and Bisby, 1). In 1900, Lindau (16) placed *Stilbum* in the Basidiomycetes near *Pilacre* including only one species *S. vulgare*. For the remaining species in the Hyphomycetes he proposed the name *Stilbella*. *Stilbum* as published by Fries (4) was unquestionably a genus of the Hyphomycetes, apparently with *S. vulgare* as the only exception among the species. It continued to be treated as such without question until 1900 with the addition of many species. Lindau's assumption that *S. vulgare* should be the type of *Stilbum*, resulting in the application of the name to a monotypic genus of the Basidiomycetes, does not appear to be justified if recommendation VI of the International Rules of Botanical Nomenclature (Cambridge revision) is followed.

STILBUM RAMOSUM Peck

In 1937, A. H. Smith collected a fungus on a lepidopterous larva near Ann Arbor. This has many of the characters of a *Stilbum*, differing principally in the branched polycephalous synnemata. In 1874, Peck (9) described a branched entomogenous fungus as *Stilbum ramosum*. His description is not sufficient to determine accurately the identity of the fungus. Through the kindness of H. D. House, the type collection in the Herbarium of the New York State Museum was loaned for study. It apparently is only

a portion of the original collection. It consists of two fragments of synnemata glued to paper (FIG. 4, A). They are 6 and 15 mm. long and 0.2–0.5 mm. thick. The synnemata have branches 6–7 mm. long with short secondary branchlets terminated by globoid to subgloboid heads, 0.3–0.8 mm. in diameter. From the fragments it is not possible to determine the arrangements of the

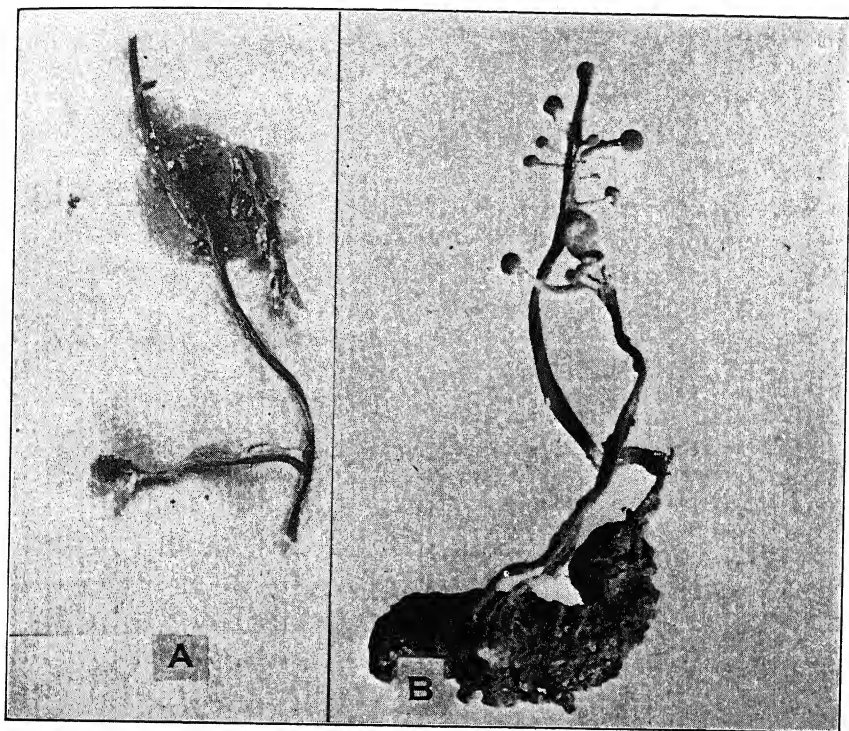


FIG. 4. *Stilbum ramosum* and *Polycephalomyces paludosus*.

branches. Peck states "sometimes creeping and sending up branches at intervals." The synnemata are now cinnamon-brown to light cream. Peck describes them as white above, pallid or brownish below. The heads are light brown and are covered by an agglutinated layer of spores. The conidia are hyaline, ellipsoid to obovoid, $2.2\text{--}3.3 \times 1.1\text{--}1.5 \mu$ and are covered by a mucus. They are produced singly on subulate phialides which are $15\text{--}30 \mu$ long and up to 1.5μ wide at the base. The phialides are closely com-

pacted forming a dense peripheral layer of the head. No part of the host was found with the type. Peck states that the fungus occurred on dead larvae of insects buried in rotten wood. This suggests that the host may be coleopterous.

Kobayasi (5) has described a somewhat similar species, *Polycephalomyces formosus*, on coleopterous larvae in Japan. His illustration shows stouter synnemata with shorter, more numerous, crowded to cespitose branches. The genus *Polycephalomyces* proposed by Kobayasi differs from *Stilbum* principally in the polycephalous synnemata, a distinction which has been used in separating other genera of the Stilbaceae. The combination ***Polycephalomyces ramosus*** (Peck) comb. nov. is proposed for *Stilbum ramosum*. The Michigan collections of Dr. Smith differ from both *P. formosus* and *P. ramosus* and a new species is proposed.

***Polycephalomyces paludosus* sp. nov.**

Synnemata capitata, 10–20 mm. longa, 0.5–0.8 mm. crassa, cinnamomea, ramosis recte dispositis, 1–4 mm. longis, 0.1–0.2 mm. crassis; partes fertiles terminales, globosae, capitulis globosis, 0.2–0.4 mm. diam., flavo-brunneis; phialides capitulorum dense dispositae, subulatae, 12–20 μ longae, deorsum 1–1.5 μ crassae, apice unicum conidium gignentes; phialides ramorum sparsae, ventricosae, sursum raro stellatae, 10.5–14.7 \times 1.5–2 μ ; conidia hyalina, obovoidea, 1.8–2.5 \times 1.1–1.3 μ , muco obducentia.

Ex larva lepidopteri, Kent Lake, New Hudson, Mich., Sept. 13, 1937, A. H. Smith (7560).

Synnemata capitate, 10–20 mm. long, 0.5–0.8 mm. thick, cinnamon-brown, branched, the branches at right angles, 1–4 mm. long, 0.1–0.2 mm. thick, the branches and the upper portions of the stems slightly pulverulent, the hyphae of the stipes 2–3.4 μ wide, thin-walled, hyaline and parallel in the interior, brownish and more or less interwoven in the outer layer; fertile parts terminating the main stem and branches, globoid, 0.2–0.4 mm. dia. including the dried layer of spores, yellowish brown, composed of hyaline, thin-walled hyphae radiating outward from the apex of the branch, repeatedly branching to form the dense peripheral layer of the conidiophores, the terminal phialides subulate, 12–20 μ long, 1–1.5 μ wide at the base, phialides occurring scattered on the branches below the heads, ventricose, occasionally stellate above, 10.5–14.7 \times 1.5–2 μ ; conidia produced singly, hyaline, obovoid, 1.8–2.5 \times 1.1–1.3 μ covered by a mucus, agglutinating (FIG. 4, B).

On a lepidopterous larva, Kent Lake, New Hudson, Mich., Sept. 13, 1937, A. H. Smith (7560-type).

This collection was found closely associated with specimens of *Cordyceps paludosa* Mains (8) indicating that this may be the conidial stage of that species.

In addition to the difference in its host, *Polycephalomycetes paludosa* has smaller conidia and more regular development of its synnemata than *P. formosus* and *P. ramosus*.

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EXPLANATION OF FIGURES

FIG. 1. *A*, *Cordyceps Puiggarii* Speg., type (*Herb. Speg. 1771*) on *Polybia fasciata* described in 1889. $\times 4$. *B*, *Cordyceps Puiggarii*, type, showing puberulent stipes and sterile apex of clava. $\times 16$. *C*, Two fragments of specimen 154 of Puiggari (*Herb. Speg. 1772*) described as *C. Puiggarii* by Spegazzini in 1919 ($= C. curculionum$). $\times 5$.

FIG. 2. *Cordyceps subsessilis* Petch, *A*. One clava of the type (*Farlow Herb. 6145*); the perithecia mostly free with a few still embedded in a soft stroma. $\times 16$. *B*. One clava of collection *Smith 7724*, the perithecia almost completely embedded in the stroma. $\times 16$. *C*. Collection *Smith 14573*, part of the perithecia embedded and part free. $\times 16$.

FIG. 3. *A*. *Cordyceps peltata* Wakefield (*Farlow Herb. 4046*) showing coalesced heads viewed from above. $\times 10$. *B*. *C. peltata*, side view of one peltate head showing short stipe. $\times 12$. *C*. *Stilbum burmense* showing bicolored synnemata arising from the head and thorax of an ant. $\times 6$.

FIG. 4. *A*. One portion of the type of *Stilbum ramosum* Peck. $\times 5$. *B*. *Polycephalomyces paludosus* Mains, type, showing polycephalous branched synnemata. $\times 3.5$.

STATUS OF THE RUST GENERA ALLO- PUCCINIA, LEUCOTELIUM, EDYTHEA, AND YPSILOSPORA¹

M. J. THIRUMALACHAR AND GEORGE B. CUMMINS

(WITH 5 FIGURES)

The following notes have resulted from a careful study of specimens of the genera *Allopuccinia*, *Leucotelium*, *Edythea*, and *Ypsilospora*. For reasons stated below three of these genera are here reduced to synonymy.

ALLOPUCCINIA Jackson and LEUCOTELIUM Tranzschel

In 1931, Jackson (5) described the genus *Allopuccinia* to accommodate a rust on *Amicia lobbiana* from San Felipe and Sorata, Bolivia (Holway 611; type). The genus was characterized as having subcuticular pycnia, subepidermal, uredinoid aecia (primary uredia) with peripheral paraphyses, and subepidermal telia with hyaline, stipitate, two-celled teliospores (FIG. 2) germinating without a rest period with the production of four-celled external basidia. The presence of subcuticular pycnia, a type not found in *Puccinia*, provided the principal character used in distinguishing *Allopuccinia* from *Puccinia*.

Sydow (10), in 1930, described the genus *Sorataea*, also on *Amicia lobbiana* collected by Rusby at Sorata, Bolivia in 1886. Uredia and telia were described. A comparison of the description of *Sorataea amiciae* Syd. with that given by Jackson for *Allopuccinia diluta* Jacks. & Holw. clearly indicates that both authors were describing the same fungus. Since *Sorataea* was published before *Allopuccinia* the latter genus becomes a synonym of *Sorataea* and *A. diluta* a synonym of *S. amiciae*.

¹Journal Paper Number 323, of the Purdue University Agricultural Experiment Station. Contribution from the Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin, and the Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana.

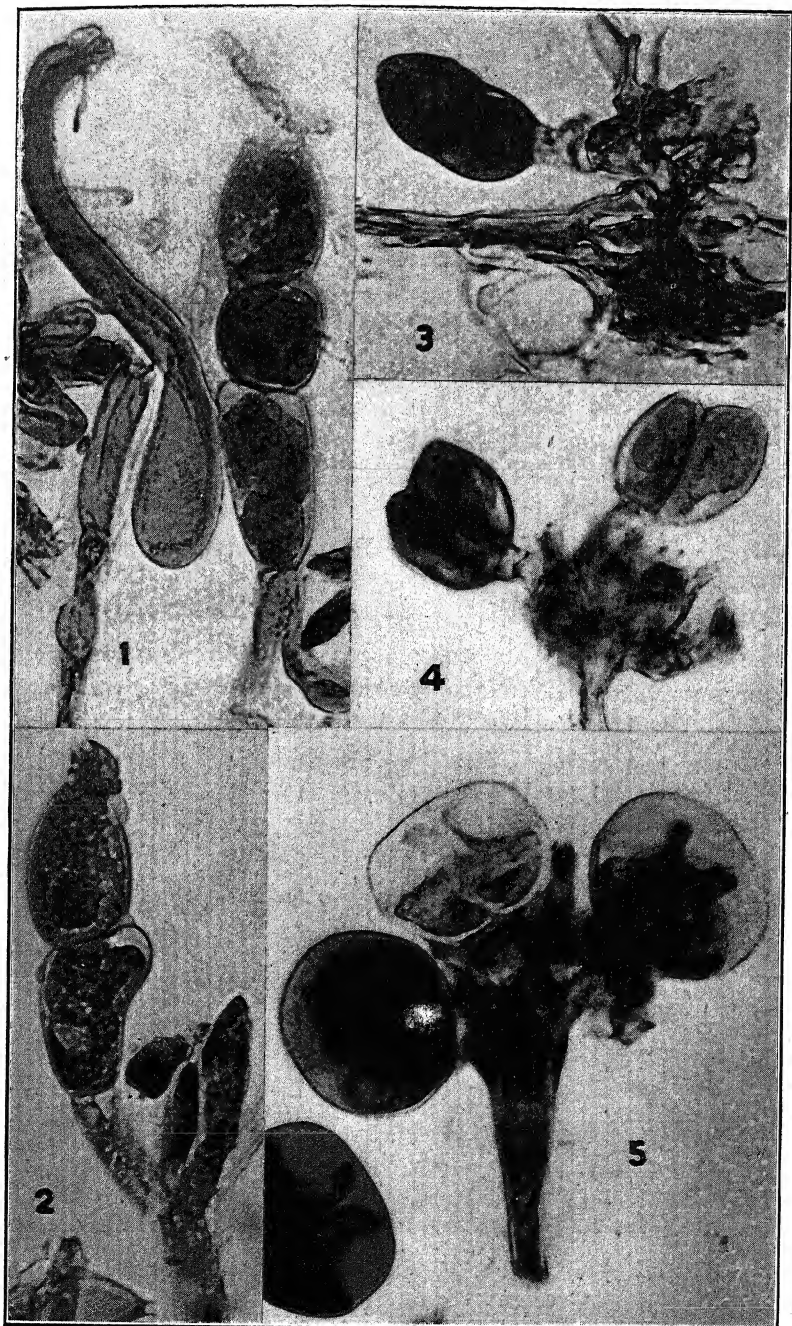
In our study of the sori we have found that, in the uredia and telia, the spores are borne in groups on basal sporogenous cells (FIG. 2), a feature not described by Sydow or Jackson but pointed out by Mains (8) in his study of the genus *Maravalia*. The presence of such basal cells has been used increasingly as a differential character in the separation of genera in the Uredinales although Kuhnholz-Lordat (6) considers, in the genus *Puccinia*, that basal cells merely represent one method of teliospore development.

The importance accorded to the formation of basal cells will largely determine the validity of the genus *Leucotelium* Tranz. (13). In *Leucotelium* the teliospores are stipitate, two-celled, colorless, and develop singly from a compact hymenial layer. The pycnia in *Leucotelium* are also subcuticular but the aecia are aecioid and the species [*L. cerasi* (Bereng.) Tranz.] heteroecious. Since the type of life cycle and the morphology of the aecia are not considered of significance in the delimitation of genera, the absence of basal cells remains as the only character separating *Leucotelium* from *Sorataea*. Until additional species are described in and more information is available concerning these two genera, it appears inadvisable to reduce *Leucotelium* to synonymy.

Three-celled teliospores (FIG. 1) have been found to occur in *Sorataea amiciae* which may indicate, as Jackson suggested (5), that *Sorataea* and *Mimema* are closely related.

EDYTHEA Jackson

Jackson (4), in 1931, described the genus *Edythea* to accommodate three South American rusts of *Berberis*, *E. quitensis* (Lagerh.) Jacks. & Holw., *E. berberidis* (Lagerh.) Jacks. & Holw., and *E. tenella* Jacks. & Holw. The principal characteristic of the genus is the presence of superficial uredia and telia, and the individual spores pedicellate from the apex of sporogenous stalks (FIG. 4) which emerge from the stomata prior to sporulation. The teliospores are nearly colorless, two-celled, mostly diorchidioid, and germinate without a rest period by the production of four-celled, external basidia. There is only a relatively small mass of hyphae beneath the stomata (FIG. 3). To quote Jackson: "There is no sorus in the usual sense of the term."



FIGS. 1-5. Spores of plant rusts.

Only a few sporogenous stalks emerge from the stomata and the stomata are not ruptured (FIG. 3). Sori of this type have been called extrastomatal by Cummins (1) and superstomatal by Mains (7). Pycnia and aecia are not known for the genus.

In 1918, the Sydows (11) described the genus *Desmella* and later Cummins (2) published an account of the morphology of the sori of *D. aneimiae* (P. Henn.) Syd. and *D. superficialis* (Speg.) Syd. The sorus in *Desmella* is superstomatal with a few sporogenous stalks which emerge from the stomata and bear, at their apices, several pedicellate spores (FIG. 5). The teliospores are diorchidioid, two-celled, and pale yellowish or hyaline. Pycnia and aecia are not known for any of the species.

It is obvious that the characters of *Edythea* duplicate those of *Desmella*. In the presence of such morphological similarity there is no justification for segregating genera on the basis of host groups, even if, as in the present case, the hosts are not closely related. Consequently we reduce *Edythea* to synonymy and propose the following transfer of species: ***Desmella quitensis*** (Lagerh.) n. comb. (*Uropyxis quitensis* Lagerh.; Arthur, Bot. Gaz. 65: 464. 1918; *Edythea quitensis* Jacks. & Holw.; Jackson, Mycologia 23: 99. 1931); ***Desmella berberidis*** (Lagerh.) n. comb. (*Sphenospora berberidis* Lagerh.; Arthur, Bot. Gaz. 65: 464. 1918; *Edythea berberidis* Jacks. & Holw.; Jackson, Mycologia 23: 99. 1931); ***Desmella tenella*** (Jacks. & Holw.) n. comb. (*Edythea tenella* Jacks. & Holw.; Jackson, Mycologia 23: 100. 1931).

YPSILOSPORA Cummins

The genus *Ypsilospora* was described by Cummins (3) in 1941 for a leguminous rust (*Y. baphiae* Cumm.) collected on *Baphia nitida* in Sierra Leone (Deighton 2138; type). The rust has subcuticular pycnia and subepidermal telia. The teliospores, described as one-celled and borne in pairs at the apex of a common pedicel, are uniformly thin-walled, hyaline, and germinate without a rest period by the production of four-celled, external basidia. Some resemblance to the genus *Sphenospora* was noted.

After a careful re-examination of the rust we question the valid-

ity of the genus. The teliospores are developed in subepidermal sori and appear to be binate. The spores are of approximately the same size and, while often closely appressed to one another, there is no common wall. In younger pairs one of the spores is obviously smaller than the other, as indicated in the original illustrations (3). Further study has also revealed that the two spores are of unlike age; one of each pair germinates before the other.

These observations indicate that the structures originally described as pedicels should be interpreted as elongated basal sporogenous cells, each bearing two sessile teliospores that are produced successively and not simultaneously. This has also been suggested as a possible interpretation by Olive (9), in connection with his studies of *Sphenospora kevorkianii* Linder. Similar elongation of a basal cell with consequent similarity of appearance to a pedicel has been pointed out by Thirumalachar (12) in *Chrysocelis ascotela* (Syd.) Thirum. This interpretation of the morphology of the telia and teliospores together with the presence of subcuticular pycnia leads us to conclude that *Ypsilospora* is synonymous with *Chaconia* Juel. Consequently, we make the following generic transfer: *Chaconia baphiae* (Cumm.) n. comb. (*Ypsilospora baphiae* Cumm. Bull. Torrey Club 68: 47. 1941) and consider the rust to be a microcyclic species of *Chaconia* in which only two teliospores, rather than several, develop on each basal cell.

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EXPLANATION OF FIGURES

FIG. 1. *Sorataea amiciae*; one paraphysis and one of the three-celled teliospores which occasionally occur in this species. FIG. 2. *Sorataea amiciae*; a typical two-celled teliospore attached to the basal cell. (Both from type of *Allopuccinia diluta* Jacks. & Holw.) $\times 800$. FIG. 3. *Desmella tenella*; free-hand section through a telium. Note the small amount of sub-stomatal mycelium, the basal cell passing outward through the stoma without rupturing the epidermis. FIG. 4. *Desmella tenella*; a single detached basal cell with two teliospores attached. (Both from type of *Edythea tenella* Jacks. & Holw.) $\times 800$. FIG. 5. *Desmella superficialis*; a single detached basal cell bearing three teliospores. (From Thaxter No. 46) $\times 975$. All preparations stained.

BASIC FUCHSIN AS A NUCLEAR STAIN FOR FUNGI¹

EDWARD D. DeLAMATER, M.D., Ph.D.²

(WITH 1 FIGURE)

Negative or weak Feulgen reactions have been obtained in many plant organisms. The three procedures for staining with basic fuchsin presented in this paper were developed in consequence of failure to obtain adequate Feulgen reactions in cytologic studies on certain fungi pathogenic for man, and because of the difficulty encountered in obtaining iron alum hematoxylin preparations which do not have a murky aspect. Clear and beautiful preparations can be obtained by the following technics, which appear to hold tremendous promise for studies of nuclear phenomena in the fungi.

Ohlmacher (1895) was the first and, we believe, the only author to use formaldehyde as a mordant for basic fuchsin staining. He applied his methods to bacteria and tissues. The studies of DeLamater and Ulrich extend, but only partially confirm, the initial observations of Ohlmacher.

A full account of the studies performed in the delineation of these procedures is presented elsewhere. These studies demonstrate that preliminary acid hydrolysis of the cells to be stained, comparable to that required in the Feulgen reaction, is necessary to increase the specificity of the stain for the nucleus. These studies also indicate that an aqueous solution of basic fuchsin produces an impermanent stain which, although extremely useful in wet mounts of tissue, fades in a short time. The stain can be made permanent, however, by (1) the mordanting of hydrolyzed cells in 1 to 4 per cent solution of formalin prior to staining or (2) by

¹ Read at the meeting of the American Association for the Advancement of Science, Chicago, Illinois, December 26 to 31, 1947.

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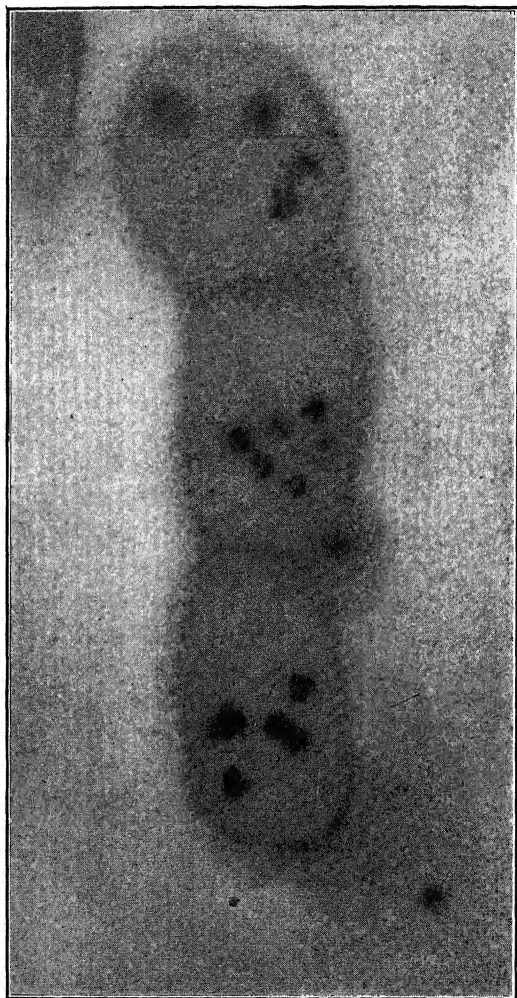


FIG. 1. *Blastomyces dermatitidis*. Cell grown at 37° C. on dextrose nutrient agar, showing early resting nuclei (formaldehyde-basic fuchsin stain, $\times 3,400$).

the combining of 2 per cent formalin with the stain. It was shown further that the acid hydrolysis is as important to the mordanting effect of the aldehyde as it is to the specificity of the stain for the nucleus. Other dyes of the phenyl methane group, such as crystal violet, in which the para-amino radicals are blocked out with methyl groups, stain, but are not mordanted with aldehyde.

MATERIALS AND METHODS

Preparation of solutions.—Basic fuchsin supplied by the National Aniline Division of the Allied Chemical and Dye Corporation with the certification number NF54 has been used. The dye was prepared as a 0.5 per cent solution in distilled water; to each 5 cc. of this solution 0.2 cc. of normal hydrochloric acid sometimes was added. The resultant solution has an approximate normality of twenty-fifth normal solution of hydrochloric acid. The dye also was prepared as a 0.5 per cent solution in twenty-fifth normal hydrochloric acid. Other solutions of basic fuchsin used will be described when the procedures are outlined. The normal hydrochloric acid for hydrolysis was prepared by dilution of concentrated (36 normal) hydrochloric acid with distilled water to the proper dilution. Alcohols of the proper dilution were prepared from 95 per cent and absolute alcohol by the addition of the proper amount of distilled water. Alcohols were used in 10 per cent stepwise dilutions. Only distilled water was used in the washing of cells after hydrolysis and after aldehyde mordanting.

Organisms tested.—Fourteen strains of *Blastomyces dermatitidis* from various sources, as well as *Candida albicans*, *C. albicans* variety *tropicalis*, *C. albicans* variety *stellatoidea*, *C. Krusei*, *C. parakrusei*, *C. guilliermondi*, *Histoplasma capsulatum* and *Saccharomyces* sp., were used as test organisms. Tissue sections from spontaneous *Blastomyces dermatitidis* infection in the dog also were used as test objects. Smears of material from mice infected with *Sporotrichum schenckii* were studied also. The most extensive studies to date have been done with *Blastomyces*.

Cultural methods.—The yeastlike phase of *Blastomyces* was induced by growing the organisms at 37° C. on a highly nutrient medium (dextrose nutrient agar). Cultures were fixed in situ with Schaudinn's fixative (Feulgen's and Zenker's fixatives were found to produce more shrinkage), suspended with a platinum loop and poured into 15 cc. centrifuge tubes. All manipulations of fungi in this phase were done in these tubes. Solutions were changed by centrifuging the cells, pouring off the old and adding the new solution. The filamentous phase of these fungi was induced at a temperature of 30° C. on a nutrient-poor medium, such

as cornmeal-extract agar or Czapek's agar in Petri dishes. Over the surface of the agar sterilized cellophane membranes cut to the size of a Petri dish were spread. The inoculation was made on top of the membranes. By regulating the amount of nutrient in the agar the thickness of the mycelial mat can be controlled. This can then be fixed in situ and manipulated through the various procedures to be described by simply transferring the cellophane membrane containing the fungus from one Petri dish to another containing the desired solution. At the time of mounting the mycelium is peeled from the membrane and mounted in pieces in clarite or balsam.

As stated all the three procedures for the use of basic fuchsin as a nuclear stain require carefully controlled acid hydrolysis comparable to that used in the Feulgen technic. A description of the three procedures follows.

Procedure 1: use of aqueous basic fuchsin.—Aqueous basic fuchsin without mordanting produces a precise, delicate and intense, red stain of the nuclei. This stain fades rapidly in water mounts, but is extremely useful for immediate direct observation. It may be made more lasting by mounting the cells in a sugar-acacia-glycerine mixture. Mounts made in this medium will last several weeks and are likewise extremely useful.

Fix cells in Schaudinn's solution	1 hour
Wash: In 30 per cent alcohol	15 minutes
In 20 per cent alcohol	15 minutes
In 10 per cent alcohol	15 minutes
In distilled water	Wash
Hydrolyze in normal hydrochloric acid:	
60° C.	10 minutes
(Optimal hydrolysis periods should be determined for each organism studied. Temperature must be accurately controlled.)	
Wash in distilled water	5 minutes

Place in a 0.25 per cent aqueous solution of basic fuchsin (not acidified) for five to fifteen minutes to stain. (If samples of cells are observed during this period, the avidity of the nuclei for the dye can be observed and the staining stopped at any desired level.)

Wash in distilled water. (Destaining occurs in the wash water. The cells can be studied immediately, on wet mounts sealed with

vaselin. If cells are too heavily stained, continued washing will eliminate the excess.)

Water menstruum for mounts.—Lee has described the following aqueous mounting medium which has been found to give excellent preparations.

Solution A

This solution contains:

50 per cent solution of glycerin	2 parts
Cold saturated solution of sugar	1 part
Cold saturated solution of gum arabic	1 part

Four dilutions of this solution of varying strengths are prepared with distilled water and the cells are run through them before being placed in the solution of full strength. Too rapid exposure to the full strength solution tends to cause shrinkage or collapse of the cells. Should too great destaining occur during processing, more of the stain can be added at any point and the cell nuclei again will take up the dye.

Solution B

This solution contains:

Picked gum arabic (or acacia)	50 gm.
Cane sugar (not candied)	50 gm.
Distilled water	50 cc.

Dissolve over a water bath and add 0.05 gm. of thymol.

This solution may be diluted with solution A, and cells may be transferred through graded mixtures of the two solutions. Such a procedure helps to prevent collapse of the cells. The walls of *Blastomyces* and other fungi appear to act as selective semipermeable membranes. This action on the part of the cell walls can be extremely bothersome in cytologic work.

Procedure 2: use of aqueous basic fuchsin with aldehyde mordanting.—The aqueous basic fuchsin stain just described can be made permanent by mordanting the hydrolyzed cells in 2 per cent solution of formalin for two to four minutes before exposing them to the stain. The outline of the procedure follows:

Fix cells in Schaudinn's solution	1 hour
Wash: In 30 per cent alcohol	15 minutes
In 20 per cent alcohol	15 minutes
In 10 per cent alcohol	15 minutes
In distilled water	15 minutes

Hydrolyze in normal hydrochloric acid:

60° C. 10 minutes

(Optimal hydrolysis periods should be determined for each organism studied. Temperature must be accurately controlled.)

Wash in distilled water 5 minutes

Mordant in 2 per cent formalin (to make up: dilute formalin, 10 per cent solution of formaldehyde, to a 2 per cent solution with distilled water) for two to four minutes. Wash in distilled water. Stain in 0.5 per cent aqueous basic fuchsin in twenty-fifth normal hydrochloric acid for fifteen minutes. (As indicated previously this can be approximated sufficiently closely and without causing too great dilution of the stain, by adding 0.2 cc. of normal hydrochloric acid per 5 cc. of 0.5 per cent aqueous solution of basic fuchsin.)

Wash in distilled water.

Run through graded alcohols. Destaining occurs gradually in alcohols and can be observed directly. Rapidity of passage through the alcohols determines the degree of destaining. Acid alcohol can be used also for destaining, but it has proved difficult to stop the effect at the desired point with material being handled in test tubes. Once the proper degree of destaining is achieved the cells can be passed more rapidly through the remaining alcohols. Destaining is stopped entirely in xylene, in which basic fuchsin is insoluble.

Clear in xylene.

Mount in balsam or clarite.

Procedure 3: use of aqueous basic fuchsin with aldehyde mordant incorporated in the staining solution.—This method differs from the preceding only in the manner in which the stain is made up and in that no individual mordanting process is required. We have found the previous method easier to control, as a precipitation may occur in the stain when this method is used (FIG. 1).

The stain is made up as follows, so that it is 0.5 per cent basic fuchsin, 2 per cent formalin in a solution of a twenty-fifth normal hydrochloric acid.

1 per cent solution of basic fuchsin 50 cc.

Formalin (10 per cent solution) 20 cc.

Normal hydrochloric acid 4 cc.

Distilled water 26 cc.

Except for the fact that the mordanting process as outlined in procedure 2 is left out because the mordant is incorporated in the staining solution, the procedure differs in no way from procedure 2. The outline of the previous procedure will suffice for both.

Counterstaining.—Fast green can be used for counterstaining if desired. It does, however, tend to cover and obscure somewhat the delicate nuclear detail observable without its use. Fast green stains cell walls intensely and aids by defining the cells.

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ROCHESTER, MINN.

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THE NUCLEAR CYTOLOGY OF BLASTOMYCES DERMATITIDIS¹

EDWARD D. DELAMATER, M.D., Ph.D.²

(WITH 5 FIGURES)

In the extensive literature pertaining to the fungi pathogenic for man and animals no reference has been found to the nuclear structure or mechanism of *Blastomyces dermatitidis*, Gilchrist and Stokes, 1898. The various articles dealing with this organism emphasize, for the most part, its morphologic characteristics (2, 3, 15).

Emmons (10) in a personal communication has admitted having observed the multinucleate status of this fungus, but has not, or had not at that time, followed up his observation. We (6, 7) recently have evaluated cytologic technics and methods usable on this and other medically important fungous pathogens, and in addition have developed new technics, at least part of the chemical mechanism of which is understood. In these extensive studies *Blastomyces dermatitidis* (fourteen strains), *Histoplasma capsulatum*, the various species of *Candida* and *Saccharomyces* sp. have been used as test organisms. Comparable staining reactions have been obtained for all of these fungi with only slight modifications of technic for each. *Coccidioides immitis* presents certain difficulties and studies of this fungus will be reported separately elsewhere.

These studies of methods confirm Emmons' observation that *Blastomyces dermatitidis* is multinucleate.

Blastomyces dermatitidis gives a weak, but definitely positive, Feulgen reaction. This reaction, other than to give a positive, specific test for the presence of desoxyribose-nucleic acid in minute amounts in the several nuclei within the cell, is not usable as a cytologic technic for the study of nuclear phenomena. The stain-

¹ Read at the meeting of the American Association for the Advancement of Science, Chicago, Illinois, December 26 to 31, 1947.

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ing effect is much too weak and delicate to permit clear-cut observations. It is sufficiently strong, however, to permit chemical studies. Demonstration of the presence of desoxyribose-nucleic acid, even in minute amounts, is, however, of importance as additional evidence that thymonucleic acid is widely distributed among the fungi. This reaction also aided in the demonstration of the multinucleate condition of the cells of *Blastomyces*.

The multinucleate condition obtaining in *Blastomyces* deserves especial emphasis. In the presence of the demonstration by Hansen (12) of the so-called dual phenomenon and the additional work by Pontecorvo (16) and others (17) on heterokaryosis, the multinucleate condition assumes double importance as a basis for further experimental study of this fungus.

The size of the nuclei in many fungi has proved a barrier to elucidation not only of their structure (1, 9) but also of the sequence of events during their division. As yet unresolved discrepancies, for example, those between the recorded observations of Guilliermond (11) and of the Lindegrens (14) on the structure and division of yeast nuclei, emphasize the difficulties due to size of the nuclei, even though new methods, such as phase microscopy, have become available.

It seems important that the mechanism of division of chromosomal elements be established in organisms in which it is intended to study variation or genetics. It also seems important to know whether the particular mechanism involved follows the general pattern which has been established for other and larger forms, or whether new mechanisms are to be found.

It is the purpose of this paper to record observations made on what appear to be the structure and sequence of events during nuclear division and growth in *Blastomyces dermatitidis*. There are many gaps in the records due primarily to the minuteness of the objects being studied. In this study both old and new technics have been used and the results obtained by each have been remarkably consistent.

ORGANISMS STUDIED

Fourteen strains of *Blastomyces dermatitidis* from various sources have been studied. These fourteen strains showed mor-

phologic differences, one from another, which will be considered at length elsewhere. The nuclear phenomena observed in each strain were, however, comparable; no differences were noted either grossly or in detail. The presentation in this article, because similar observations were made on all fourteen strains, consists of a composite; the drawings (FIGS. 1-2) and photographs (FIGS. 3, 4 and 5) have been made from several of the strains.

METHODS

The organisms were grown in the yeastlike phase at 37° C. on dextrose nutrient agar. Cultures were fixed in situ, stirred into suspension with a platinum loop and poured into 15 cc. test tubes. All procedures on this phase of growth were carried out in these tubes. At the time of mounting, cells were mixed in the mounting medium, a drop of which was placed on a slide and a cover-slip was added.

Growth in the filamentous phase was handled in two ways. In the first, cultures were grown on Czapek's or cornmeal extract agar at 30° C. Growth on these media is thin but ordinarily an abundance of conidia and other structures are produced.

Such cultures were fixed in situ and blocks of agar were cut out and carried to water. From these the surface growth was carefully sliced with a sharp scalpel. These thin slices of agar containing the growth on their upper surfaces were stained with iron alum hematoxylin stain and mounted upright in balsam on slides. Such procedures were carried out in Petri dishes.

The second procedure consisted of growing the organisms on cellophane sheets cut to the size of Petri dishes, sterilized and placed on the surface of dextrose nutrient agar, cornmeal extract agar or Czapek's agar. These cultures were incubated at 30° C., fixed in situ and the colonies transferred to fresh Petri dishes. All staining procedures were carried out on these colonies in Petri dishes. At the time of mounting the mycelium was removed from the sheet and mounted upright on a slide in balsam or clarite.

By both these procedures the normal relationships of fungous structures could be maintained in preparations in which the cytologic picture could be easily studied.

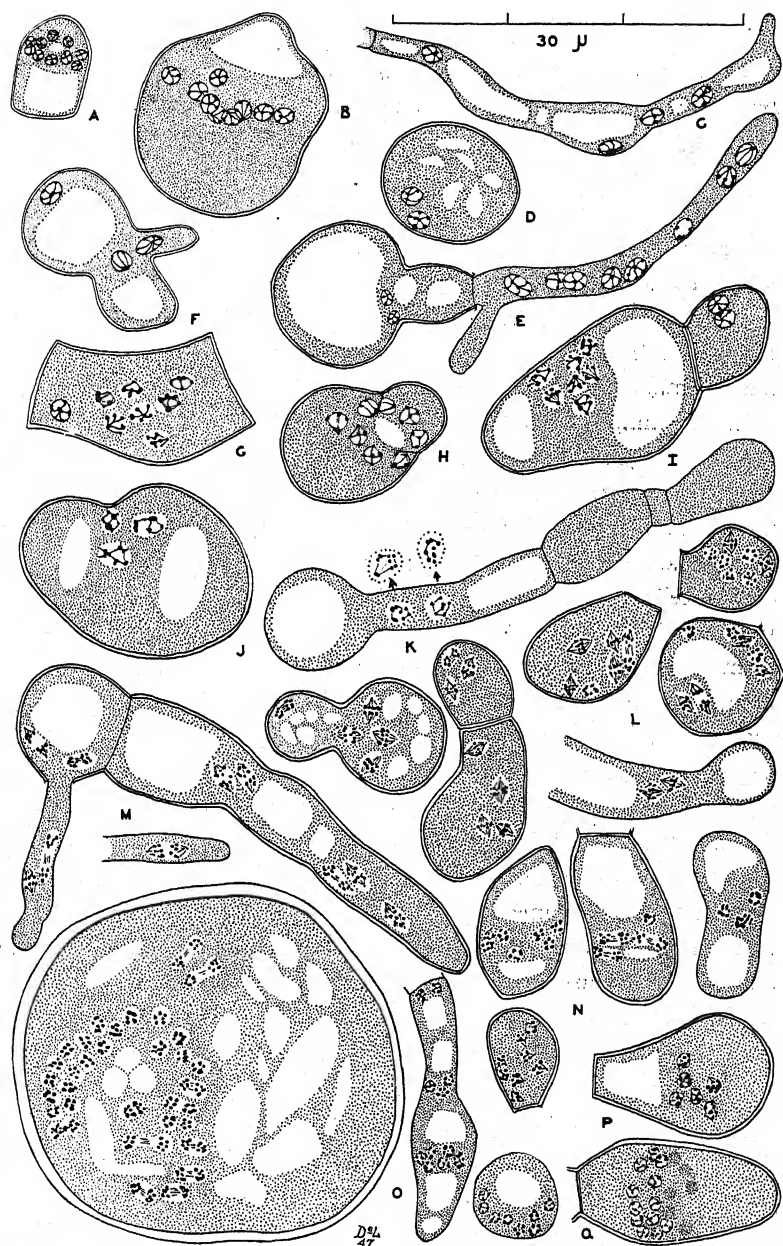


FIG. 1. *Blastomyces dermatitidis*.

FIXATION

Fixation was done with Schaudinn's solution at 60° C. Feulgen's and Zenker's fixatives were found to produce too great shrinkage.

STAINING PROCEDURES

The first procedure used consisted of an iron alum hematoxylin stain in which destaining was accomplished with a saturated solution of picric acid. Destaining was stopped by washing in water to which a drop or two of ammonium hydroxide were added. Iron alum hematoxylin preparations, however, have a cloudy aspect due to the staining of cytoplasmic elements which obscure the nuclei.

The Feulgen stain was carried out in the classic manner. The reader is referred elsewhere (6, 7) for details of the procedures used both with this and the following staining procedures.

The basic fuchsin stain was found to require acid hydrolysis of the cells similar to that necessary for the Feulgen reaction. Following hydrolysis, the cells were mordanted in 2 per cent formalin for two to four minutes, washed and then stained ten minutes in 0.04 normal hydrochloric acid which contained basic fuchsin in a concentration of 0.5 per cent (0.25 per cent may also be used). Destaining was accomplished in the alcohol-water mixtures during dehydration. Direct observation of the degree of destaining was made at each step of the procedure and the length of exposure of the cells to the alcohols was adjusted accordingly.

Tissues of experimentally infected mice and from a dog which had spontaneous blastomycosis were stained by the routine iron alum hematoxylin-eosin method, by the formaldehyde basic fuchsin method, and with the Feulgen stain.

Since the major portion of these observations were made, confirmatory studies using a new staining procedure which requires special methods have been made. This procedure will be reported in detail elsewhere (5).

CULTURAL METHODS

The yeastlike phase of *Blastomyces* was grown on dextrose nutrient agar at 37° C. For complete study of this phase, cultures

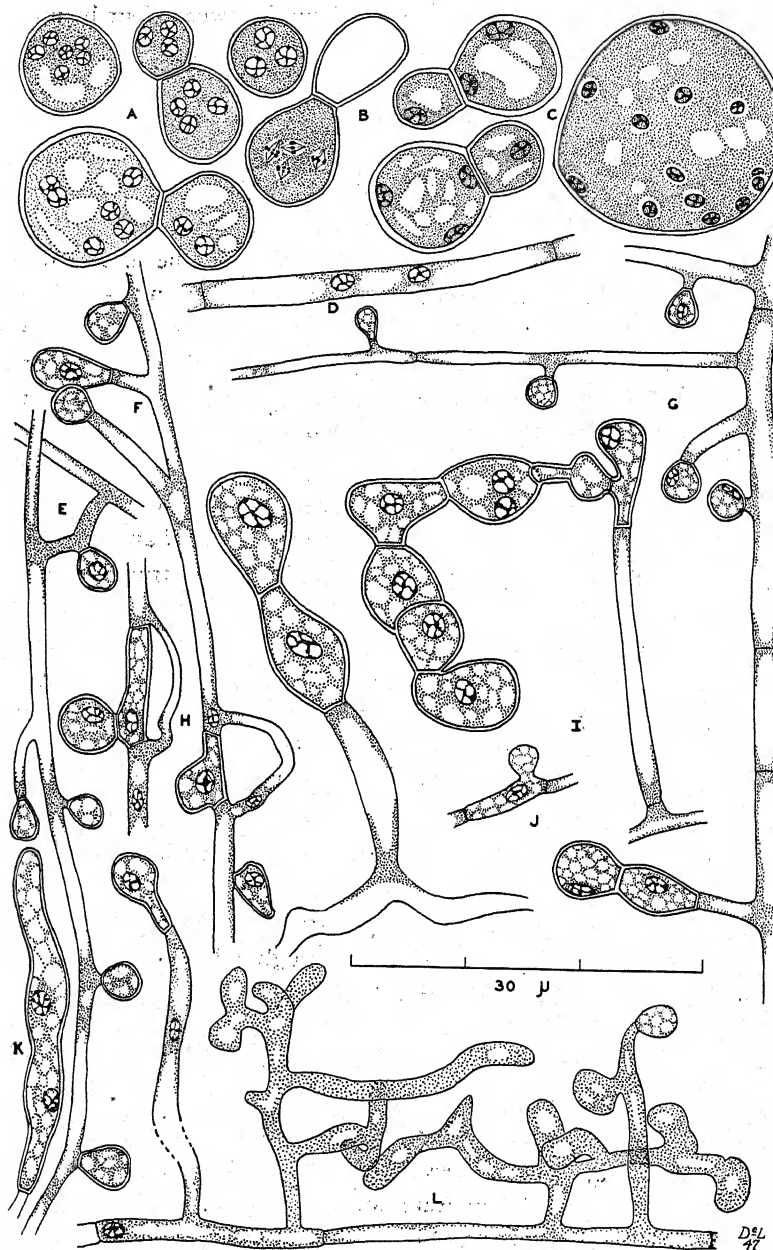
of all fourteen strains were fixed every hour for twenty-four hours. The filamentous phase was grown on cornmeal agar or Czapek's agar and on modified Sabouraud's agar at 30° C., or on cellophane membranes laid on the surface of the agar.

OBSERVATIONS

The yeastlike phase.—When grown at 37° C. on several media *Blastomyces* converts to a yeastlike growth. Levine and Ordal (13) have considered temperature, moisture and pH, in that order, the most important factors which operate in this conversion. To these must be added the inherent characteristics of the strain under observation. This last factor has been found to be as important as the others and is at present being studied.

The organism in the yeastlike phase grows in two forms with various intergrades. In the most common form the cells are elongated and suggest abortive hyphal elements (FIG. 3, *l* and *p*). In the second the cells are round or oval and simulate true bud-cells more closely (FIG. 5 *a*). The latter type simulates most closely that seen in tissue (FIG. 2, *A* and *B*).

Distribution of nuclei within the cells.—All cells, with rare exceptions, are multinucleated. In the majority of cells the nuclei occur as clusters or ringlike groups within cytoplasmic concentrations surrounding central vacuoles (FIG. 1, *A*, *B*, *H*, *L*, *M* and *Q*). More rarely they appear more evenly distributed throughout the cells and occur in the cytoplasmic strands separating vacuoles (FIG. 4, *a*, *c* and *d*). Frequently they occur in groups at the tips of cells but more often in the central part. The numbers of nuclei vary per cell. There is, however, a rough relationship between the size of the cell and the number of nuclei it contains. No nuclear counts and cell measurements have been made as yet. Nuclei have not been observed to divide into new cells or buds, but to migrate during their resting stages (FIGS. 1, *E*, *F* and *M* and 4*b*). There seems to be no immediate direct relationship between nuclear division and formation of new cells. When a new bud is partially formed, resting nuclei migrate into it. Budding occurs by the rupture of the outer wall and extension of the protoplast and inner wall through the aperture. The frayed edge of the outer wall is usually visible (FIG. 1, *E* and *M*).

FIG. 2. *Blastomyces dermatitidis*.

The filamentous phase.—In the filamentous stage grown at 30° C. or less on a variety of media the fungus forms a typical filamentous mycelium (FIG. 2, *D* to *L*).

The cells of the filamentous phase are likewise multinucleated. There is, or appears to be, no constant positional arrangement of nuclei, except that actively growing hyphal tips contain greater numbers (FIG. 1, *E*). The number of nuclei per cell also varies within wide limits.

The septations appear to be perforated. Odd shunt tubes are to be observed when solid cross walls have been laid down (FIG. 2, *H*). Hyphal fusions are common (FIG. 2, *E*).

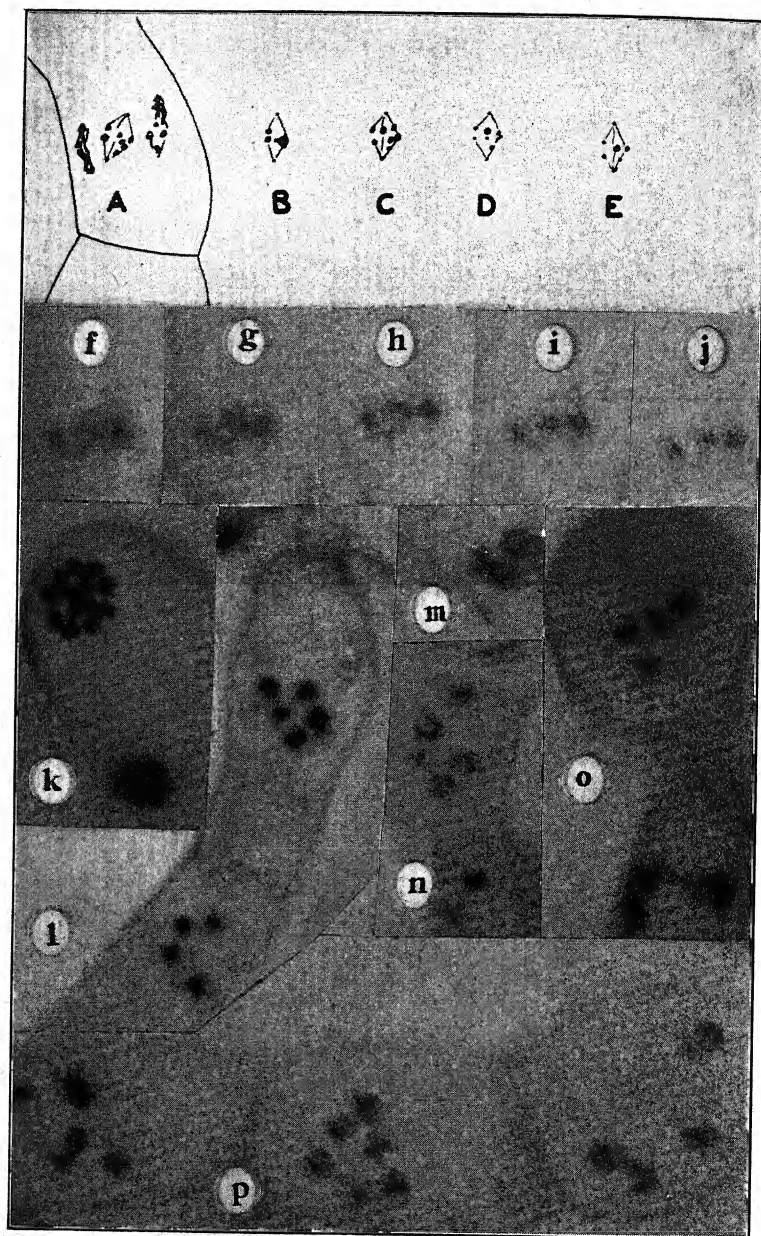
Conidia are produced in the filamentous phase. These vary widely in length from one to ten or more microns but have a fairly constant pyriform shape. They are relatively thick-walled. The walls are smooth. Odd angular forms, however, are also to be observed (FIG. 2, *E* to *K*), and chains of cells of a sporelike character occur (FIG. 2, *I*). The number of conidia varies markedly from strain to strain.

It has been considered by DeMonbreun (8) that the yeastlike phase originates from the filamentous phase by conversion through chlamydospore-like masses which reproduce. Further evidence for such a mechanism has been observed for the round cell type (4). The abortive hyphal element type of yeastlike growth appears, however, to be more closely related to true filamentous growth.

Sexuality.—No evidence, either morphologic or nuclear, for the occurrence of sexuality in *Blastomyces dermatitidis* has yet been observed.

The nuclear cycle.—The nuclear phenomena appear to be related strictly to the vegetative growth. Nuclear division and the formation of new cells appear to be two distinct and separate processes. Nuclear division occurs independently of budding. New cells appear to obtain their nuclei by migration of vegetative or resting nuclei into them (FIG. 1, *E*, *F* and *M*).

Following what appears to be a bona fide telephase the chromatinic granules or chromosomes form a dense mass (FIGS. 1, *O*, 3, *k* and *l*). These separate and are observed as discrete gran-

FIG. 3. *Blastomyces dermatitidis*.

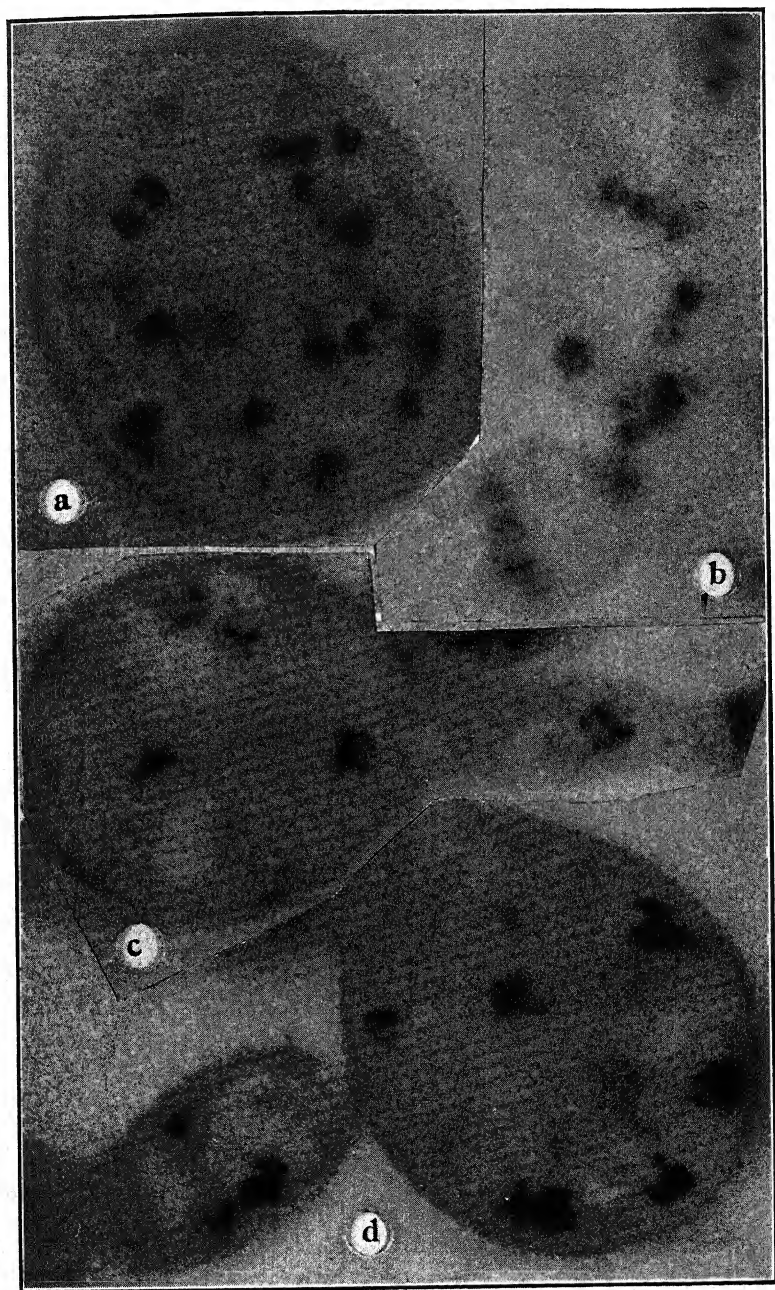


FIG. 4. *Blastomyces dermatitidis*.

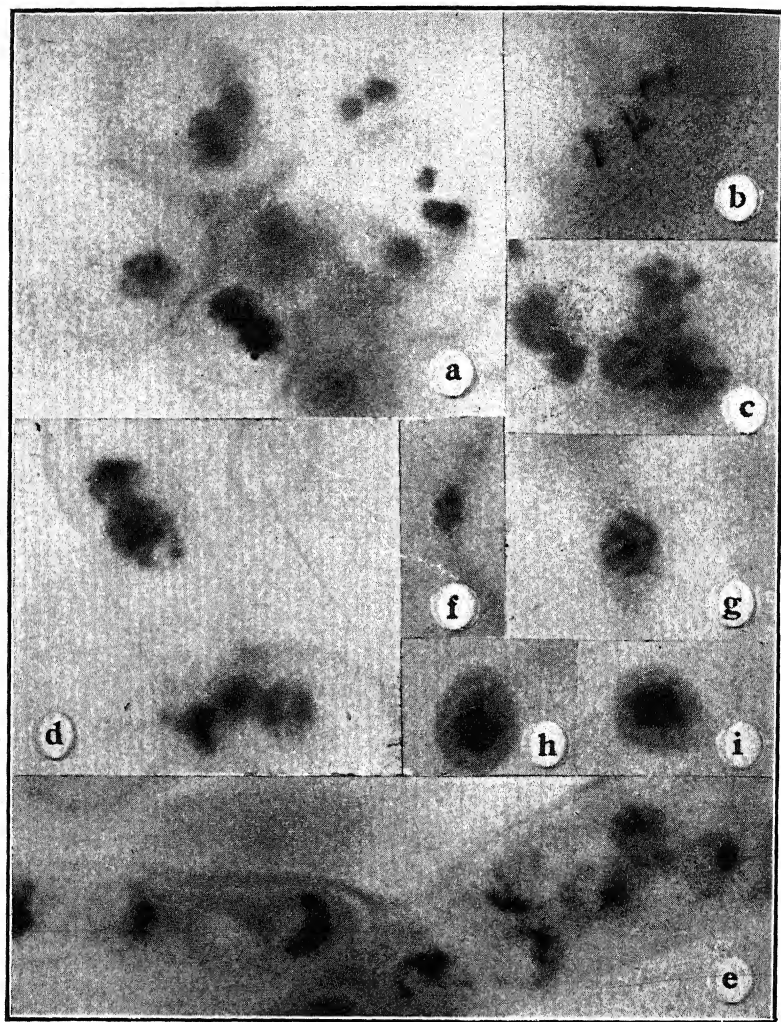


FIG. 5. *Blastomyces dermatitidis*.

ules which are integral parts of delicate threads (FIGS. 1, *P*, *Q* and *A*, and 3, *m* to *p*).

These minute resting or vegetative nuclei then undergo progressive enlargement (FIGS. 1, *A* to *F*; 3, *k* to *p*, and 5, *a* to *d*). All the nuclei within a single cell appear to be in the same stage of development, but nuclei within adjacent cells of the same cell

clump may be in distinctly different stages (FIGS. 4*b* and 5*a*). The granules become larger and the delicate linin net connecting them becomes more distinct. Frequently a darker mass or ringlike structure suggestive of a nucleolus is visible (FIG. 5, *c* and *d*).

Observation is frequently impeded by the delicacy of the structures under observation and by the large numbers of individual nuclei present, overlapping of which causes confusion. Late resting nuclei may reach a considerable size.

The stages between what has been interpreted as early prophase and metaphase are not entirely clear. From what it has been possible to observe, however, the following sequence of events seems likely. The chromatinic threads condense and appear to shorten (FIGS. 1, *G* to *J*, and 5*e*). They then aggregate at one side of the nucleus and a granule appears opposite this mass (FIG. 5*e*). This granule enlarges and becomes double (FIG. 1, *J* and *K*). The next stage observed with assurance is the metaphase (FIGS. 1, *L* and *M*, and 3, *A* to *j*). It is, however, thought that these two granules separate and migrate to the opposite poles, and that the chromatinic granules (chromosomes) line up between them.

The chromosomes or chromatinic granules become double (split?) and are drawn to the two poles (FIGS. 1, *L*, *M* and *N*, and 4*a*), forming dense masses. The whole cycle then is repeated as described.

The difficulties encountered in making observations on such minute structures are manifold. The presence of more than one nucleus per cell is confusing.

The minute size of the chromosomes prohibits an accurate count. There appear to be four optically resolvable granules. Whether these six granules represent the actual number of chromosomes in *Blastomyces* is not certain.

SUMMARY AND CONCLUSIONS

The nuclear cytology of *Blastomyces dermatitidis* has been described. Comparable results were obtained with iron alum hematoxylin stain and with basic fuchsin stain mordanted with formaldehyde. Similar results were obtained with a new and as yet unreported staining procedure.

From the observations reported it would appear that *Blastomyces* undergoes vegetative nuclear division in a manner comparable to other organisms.

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DESCRIPTION OF FIGURES

FIG. 1. *Blastomyces dermatitidis*. Drawings made with aid of a camera lucida. Cells drawn from cultures grown at 37° C. on dextrose nutrient agar. A. Small bud-cell showing ten nuclei in early resting stage arranged in cytoplasmic bridgelike concentration about a central vacuole. B. Larger bud-cell showing nuclei in a similar cluster; nuclei are larger than in A and linin net pattern with chromosomes is more distinct. C. Abortive hyphal type of growth showing numerous vacuoles and larger resting nuclei than in B. Larger dark masses within nuclear matrix are interpreted as being nucleoli. D. Bud-cell with two large resting nuclei in which linin net

and chromosomes are obvious. *E.* Cell giving rise to abortive hyphal element. Two small resting nuclei are present in mother cell; many large resting nuclei are present in abortive hyphal cell. Branch of latter shows no nuclei. *F.* Cell developing bud into which large resting nucleus is migrating. *G.* Bud-cell showing many nuclei in same stage. Chromatin masses are beginning to aggregate and become dense. This stage is interpreted as an early prophase. *H.* Bud-cell showing nuclei about vacuole in slightly earlier stage of chromatin condensation than in *G.* *I.* Bud-cells showing characteristic double wall at attachment. The smaller cell contains two large resting nuclei. The large cell contains seven nuclei in prophase in which there appears to be a linkage of all chromatin masses to a single slightly larger granule. This figure shows that all nuclei within a single cell are in the same phase or stage of division, but that adjoining cells may have nuclei in different stages. *J* and *K.* Bud-cell and abortive hyphal element containing large nuclei in a stage interpreted as further advanced into prophase than the nuclei in the large cell in *I.* The excerpts in *K* show a lower focal level and a suggestion that the large granule to which the others are tied is dividing. Stages between this and the metaphase have been difficult to follow due to the small size and complexity produced by the presence of many nuclei. *L* and *M.* Bud-cells and abortive hyphal elements showing stages interpreted as metaphase and early telephase. The chromosomes are lined up on a plate or appear to be separating. *N.* Bud-cells containing stages interpreted as late telephase clusters of granules widely separated with suggestion of lines between. *O.* Giant round cell. Such cells are common in many strains and also occur in tissue. Numerous nuclei in stages interpreted as late telephase present. *P* and *Q.* Bud-cells and abortive hyphal elements containing stages interpreted as nuclei reorganizing into resting or vegetative nuclei. The chromosomes are dense and appear to be organizing into nuclei comparable to those in *A* at which point the cycle repeats.

FIG. 2. *Blastomyces dermatitidis* in tissue, *A* to *C*; grown on cornmeal extract agar at 30° C., *D* to *L.* *A.* Bud-cells in tissue showing resting nuclei of different size comparable to those observed in cultures grown at 37° C. *B.* Metaphase stages observed in one cell. *C.* A giant cell with many resting nuclei observed in tissue. *D.* Single cell of hypha showing two large nuclei in late resting stage in cytoplasmic condensations within hypha. *E.* Hypha bearing several pyriform conidia. The nuclei in these stained too densely to show detail. A hyphal fusion is also present from which a uninucleate conidium has arisen. *F.* Hypha showing additional conidia in one of which the nucleus could be visualized. Lower on the same filament at *H* there are intercalary conidia or chlamydospores, the walls of which apparently prevent cytoplasmic flow as tubes which are interpreted as shunts have formed, circumventing them. *G.* Additional hypha showing branching and septate condition. Conidia are uninucleate. A bicellular spore is shown arising from the lower end of an hypha. *H.* See *F.* *I.* Large uninucleate and binucleate cells in chains suggestive of aberrant conidia or chlamydospores. *J.* Thin-walled uninucleate cells interpreted as early stage in development of intercalary conidium. (See *H.*) *K.* Very long aberrant conidial or chlamydospore form containing two resting nuclei. *L.* Odd, dense,

contorted filaments, the function of which is not clear. Nuclei could not be visualized due to dense cytoplasmic staining.

FIG. 3. *Blastomyces dermatitidis* grown on dextrose nutrient agar at 37° C. (basic fuchsin stain with formalin mordant). *A*. Abortive hyphal element showing three nuclei in division. The central figure is interpreted as in metaphase in longitudinal section ($\times 3,500$). *f*. Same as *A* at about the same focal level ($\times 3,400$). *B*. Same as part of *A* at a high focal level showing three resolvable masses ($\times 3,500$). *g*. Same figure as *B* at same level showing three resolvable masses ($\times 3,400$). *C*. Same figure as *B* at a focal plane 0.25μ lower than *B* showing several masses ($\times 3,500$). *h*. Same figure as *C* at same level showing increasing complexity of figure ($\times 3,400$). *D*. Same figure at a focal plane 0.25μ lower than *C* showing decrease in size of individual bodies ($\times 3,500$). *i*. Same figure as *D* at about the same focal level ($\times 3,400$). *E*. Same figure at a focal plane 0.25μ lower than *D* showing further diminution in number and mass of bodies ($\times 3,500$). *j*. Same figure as *E* at about same level ($\times 3,400$). *k*. Many dense early resting nuclei concentrated in tip of bud-cell ($\times 3,400$). *l*. Localization of nuclei in central portion of cells in abortive hyphal element. Nuclei slightly larger than in *k* ($\times 3,400$). *m*. Early resting nuclei showing breakup of dense chromatin mass into discrete chromosomes on linin net ($\times 3,400$). *n* and *o*. Further stages of same ($\times 3,400$). *p*. Abortive hyphal element showing clumping of nuclei in central portion of cells. Nuclei have further assumed characteristic resting pattern. All nuclei in a single cell appear to be in same stage.

FIG. 4. *Blastomyces dermatitidis* in yeastlike phase grown on dextrose nutrient agar at 37° C. (basic fuchsin stain with formalin mordant). *a*. Giant cell with many nuclei. Pair in upper left hand portion are regarded as being in late telephase ($\times 3,400$). *b*. Cluster of bud-cells and abortive hyphal elements showing multinucleate condition and nuclei in different resting stages in adjoining cells ($\times 3,400$). *c* and *d*. Two focal levels of same giant cell showing multinucleate condition. All nuclei in same stage, interpreted as in moderately advanced resting stage. Cell in left lower corner shows torn outer wall from separation of cells ($\times 3,400$).

FIG. 5. *Blastomyces dermatitidis* grown on dextrose nutrient agar at 37° C. and stained with basic fuchsin with formalin mordant. *a*. Cluster of budding cells showing nuclei in various resting stages from very early to late in which there are linin net and chromosomes, as well as a dark central body which may be a nucleolus ($\times 3,400$). *b*. Medium resting stage showing linin net ($\times 3,400$). *c*. Four large nuclei in late resting stages in which linin net is apparent and nucleolus-like body appears as a ring ($\times 3,400$). *d*. Stage in abortive hyphal elements similar to *c* ($\times 3,400$). *e*. Stage interpreted as early prophase in which chromosomes have aggregated to one side of nucleus opposite a faint granule ($\times 3,400$). *f*. Distorted resting stage in hypha (grown on cornmeal extract agar at 30° C.) suggesting telephase. *g*. Late resting stage in large hyphal element, with central nucleolus. *h* and *i*. Uninucleate conidia borne on filamentous stage grown at 30° C.

SOIL PHYCOMYCETES FROM BIKINI, ENIWETOK, RONGERIK AND RONGELAP ATOLLS

F. K. SPARROW¹

(WITH 19 FIGURES)

One of the happier aspects of the late world conflict has been the occasional opportunity given trained scientific personnel since cessation of hostilities to visit certain areas ordinarily inaccessible to them. Particularly is this true of biological work that has been done in the Marshalls. Located as they are in the mid-Pacific, it is uncertain how soon, if ever, a privately financed scientific expedition could have carefully investigated this remote group of atolls.

Rogers (1947) has recently given a review of the very scanty literature dealing with Marshallese fungi and notes that prior to his own collections made in 1946 through the cooperation of the U. S. Navy and the University of Hawaii, only 16 species of fungi had heretofore been reported. His first paper lists 34 species, with a promise of more to come. Of these, *Glaziella aurantiaca* was a Phycomycete belonging to the Endogonales, the others being Myxomycetes, Basidiomycetes and Fungi Imperfecti.

The present study of soil Phycomycetes from this area was made possible through the kindness of Prof. W. R. Taylor, a member of the technical staff of Joint Task Force One engaged in "Operations Crossroads." A series of 44 soil samples was obtained from Bikini, Eniwetok, Rongerik and Rongelap Atolls in the northern Marshalls. The samples were placed in small boxes and thoroughly sealed at the time of collection to prevent mixture and sent in small lots by plane to Ann Arbor. There, they were used in the preparation of gross water cultures and baited with bits of boiled

¹ Contribution from the Botany Department, University of Michigan No. 882.

grass, hemp seed, cellophane, etc., the usual method of obtaining phycomycetous fungi.

The samples were all collected at the surface of the ground and usually in thickets or coconut groves where there was some organic matter mixed with the ground and weathered coral. In those from Bikini Atoll, twelve were collected before the two blasts and eight afterwards. The post-blast samples were all from sites facing the target area. One, from Romurikku I., was taken from just above the wave line produced by the second atom bomb.

Because of the origin of these atolls and their remoteness from continental land masses, there seemed little likelihood of recovering

TABLE I
RESULTS OF SEARCH FOR PHYCOMYCETES IN SOILS FROM CERTAIN
ATOLLS OF THE MARSHALL ISLANDS

Bikini			Eniwetok		Rongelap		Rongerik	
Island	Fungi Recovered		Island	Fungi Recovered	Island	Fungi Recovered	Island	Fungi Recovered
	Pre-blast	Post-blast						
Bikini* (7)**	- +	- +	Bogon (2)	+ -	Rongelap (4)	- +	Bock (1)	+
Namu (3)	+	-	Aaraanbiru (1)	+	Rigonman (1)	-	Latoback (1)	-
Enyu (4)	+	+	Aomon (2)	+	Busch (1)	-	Enyvertok (1)	+
Romurikku (2)	-	+	Japtan (2)	+	Summary			
Entirikku (1)	+		Jieroru (1)	-	Bikini Atoll	Soil Collections	Fungi Recovered	
Chieerete (1)	-		Igurin (1)	+	Preblast Postblast	12 8	5 4	
Airukiraru (1)	-		Eleugelab (1)	+	Eniwetok Atoll	15	9	
Ourukean (1)		+	Runit (2)	+	Rongelap Atoll	6	2	
			Rujoru (1)	+	Rongerik Atoll	3	2	
			Giriinien (1)	-	Total	44	22	
			Bogombogo (1)	-				

* A plus sign indicates the presence of phycomycetous fungi; a minus sign, their absence.

** Number of soil samples.

from them such delicate fungi as Phycomycetes. In the preceding table are summarized the results of this search.

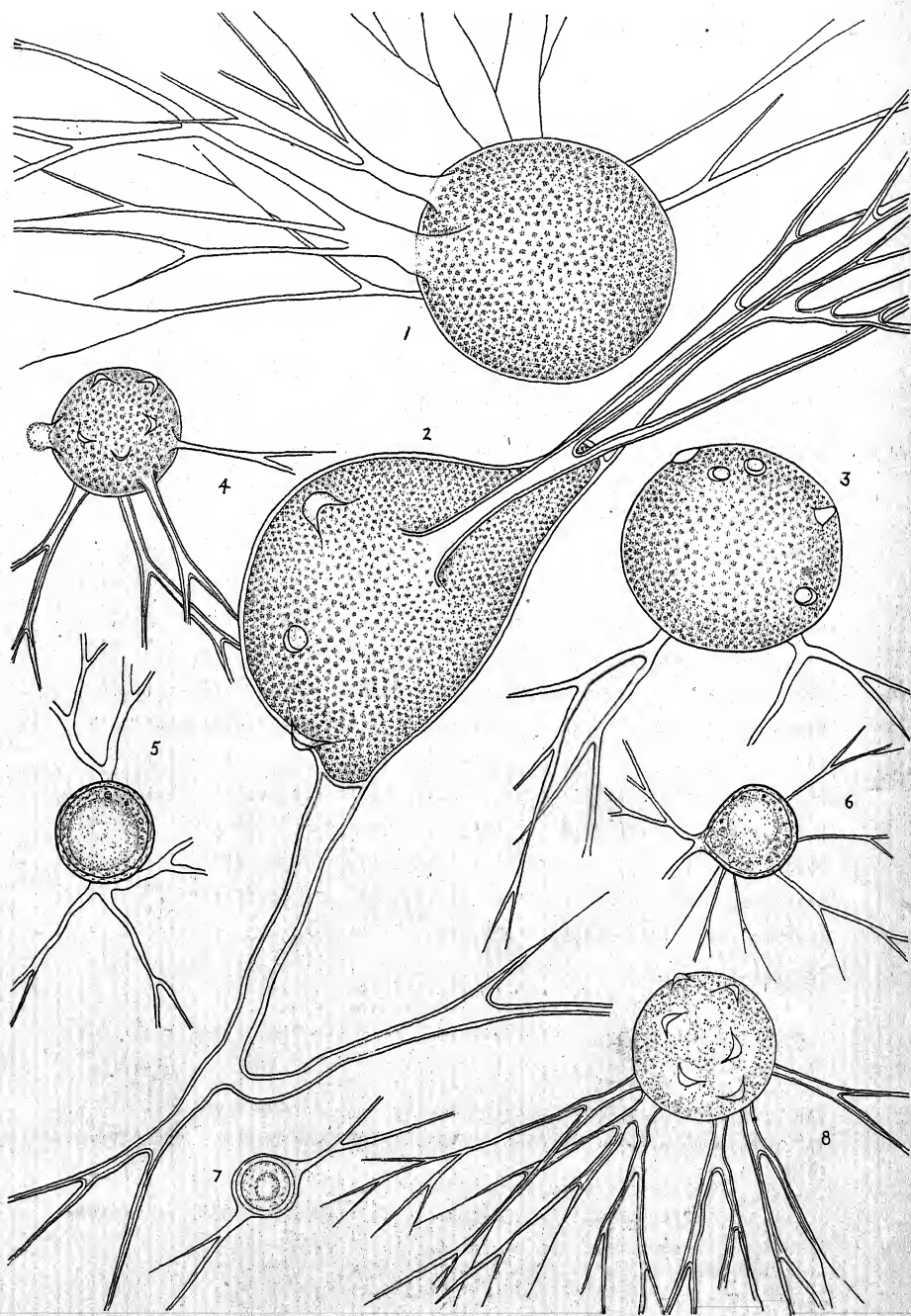
From past experience in investigating tropical soils using similar methods, it was expected that if phycomycetous fungi were present they would be such groups as the Chytridiales, Saprolegniales, Blastocladiales, Monoblepharidales and *Pythium*. No filamentous Phycomycetes, however, were recovered. Of those found in twenty-two soil samples, twenty were species of the chytrid genus *Rhizophlyctis*, the other two were chytridiaceous parasites of these species.

FUNGI RECOVERED

Rhizophlyctis spp. Three general groups of sporangial types belonging to this genus were recovered. Owing, however, to the present confused status of the species and, indeed, of our understanding of *Rhizophlyctis*, it seems better at the moment simply to characterize the groups until such time as specific and generic concepts become more clearly defined.

The two groups most commonly present both possess light amber colored mature sporangia (near "xanthine orange"²). In one they are somewhat spherical, 50-80 μ in diameter, and bear moderately coarse, richly branched rhizoids (FIGS. 4, 8). Members of this group occurred most frequently and were found on one or more islands of all the atolls except Rongerik. The other group forms spherical or irregularly ellipsoidal sporangia 115 μ or more in largest diameter and bears exceedingly stout (up to 25 μ in width), and extensive, much branched rhizoids (FIGS. 1-3). Members of this group were found only in soil from Japtan I. of Eniwetok Atoll and Bock I. of Rongerik Atoll. The zoospores formed by both groups are essentially alike, being ellipsoidal to spherical, 3 μ in diameter, and bearing several minute droplets. They emerge from few to many stout, short discharge tubes after the discharge and deliquescence of a gelatinous bubble. The first emerged spores usually form a coherent mass which soon breaks up, and further discharge is the result of flagellar action. Several

² Color terms within quotation marks are taken from R. Ridgway, Color Standards and Color Nomenclature, Washington, D. C. 1912.



FIGS. 1-8. Phycomycetes from Marshall Islands.

types of ellipsoidal or spherical resting spores have been observed associated with the amber-colored sporangia (FIGS. 5, 6). The spherical ones are $30\text{--}35\ \mu$ in diameter, the ellipsoidal $35 \times 40\ \mu$. Both have a thickened, nearly smooth brownish wall and brownish contents within which are a large oil globule and several smaller ones.

The members of these two groups closely approximate plants which have been ascribed to *Rhizophlyctis* (*Karlingia*) *rosea* by Ward (1939), Johanson (1944) and Karling (1947) and to *Entophlyctis aurea* by Haskins (1946). The coloration, however, is certainly not "pink." It should be noted that the color of the cytoplasm of *R. rosea* has been stated by Karling (1947) to vary from rosy pink or light orange to golden.

Color is emphasized here because of its bearing on the third of the three groups of species found. Members of this group occurred intermixed with the amber-colored forms and were recovered from Ourukeen I., Bikini Atoll, Igurin I., Eniwetok Atoll and Rongelap I., Rongelap Atoll. Several well marked features distinguished them. Although the sporangia were occasionally spherical (FIG. 9) more often they were irregularly angular due primarily to the strongly flaring bases of the broad discharge tubes (FIGS. 10, 11). The rhizoids were never so broad as in the amber-colored forms and, most striking of all, the contents were shot through with red granules (near "Carmine Red" and "Nopal Red"). The zoospores were colorless and little different in size and shape from those previously described. The several granules in them were exceedingly minute.

Olpidium Rhizophlyctidis n. sp.

Sporangia subglobosa vel ellipsoidea, $25\text{--}50\ \mu$ longa, diametro $12\text{--}45\ \mu$, hyalina, leviter circumvallata, tubo latiusculo singulo praedita ad exteriorem; zoosporis numerosis, globosis, ca. $2\ \mu$ diametentibus, absque, globulo conspicuo, sed flagellum longum posteriorem ferentibus, erratico motilibus motu interdum saltante interdum natante; sporis perdurantibus sphaericis, $12\text{--}20\ \mu$ diametro, pallide brunneis, ut videtur asexualiter formati, pariete laevi ca. $2\ \mu$ crassa, uniguttulata. Germinatio mihi ignota.

Parasiticum in thallis sporangiisque generis *Rhizophlyctidis* ad insulas Bikini, Eniwetok et Rongelap, in Insulis Marshallianis.

Sporangia spherical or ellipsoidal, $25\text{--}50\ \mu$ long by $12\text{--}45\ \mu$ in diameter, hyaline, smooth-walled, with a single discharge tube

which protrudes through the host wall; zoospores numerous, spherical, about 2μ in diameter, without a conspicuous globule, posteriorly uniflagellate, movement an erratic hopping interspersed with periods of swimming; resting spore spherical, $12-20\mu$ in diameter, faintly brown, apparently asexually formed, with a smooth wall about 2μ thick, contents with a large fat droplet, germination not observed.

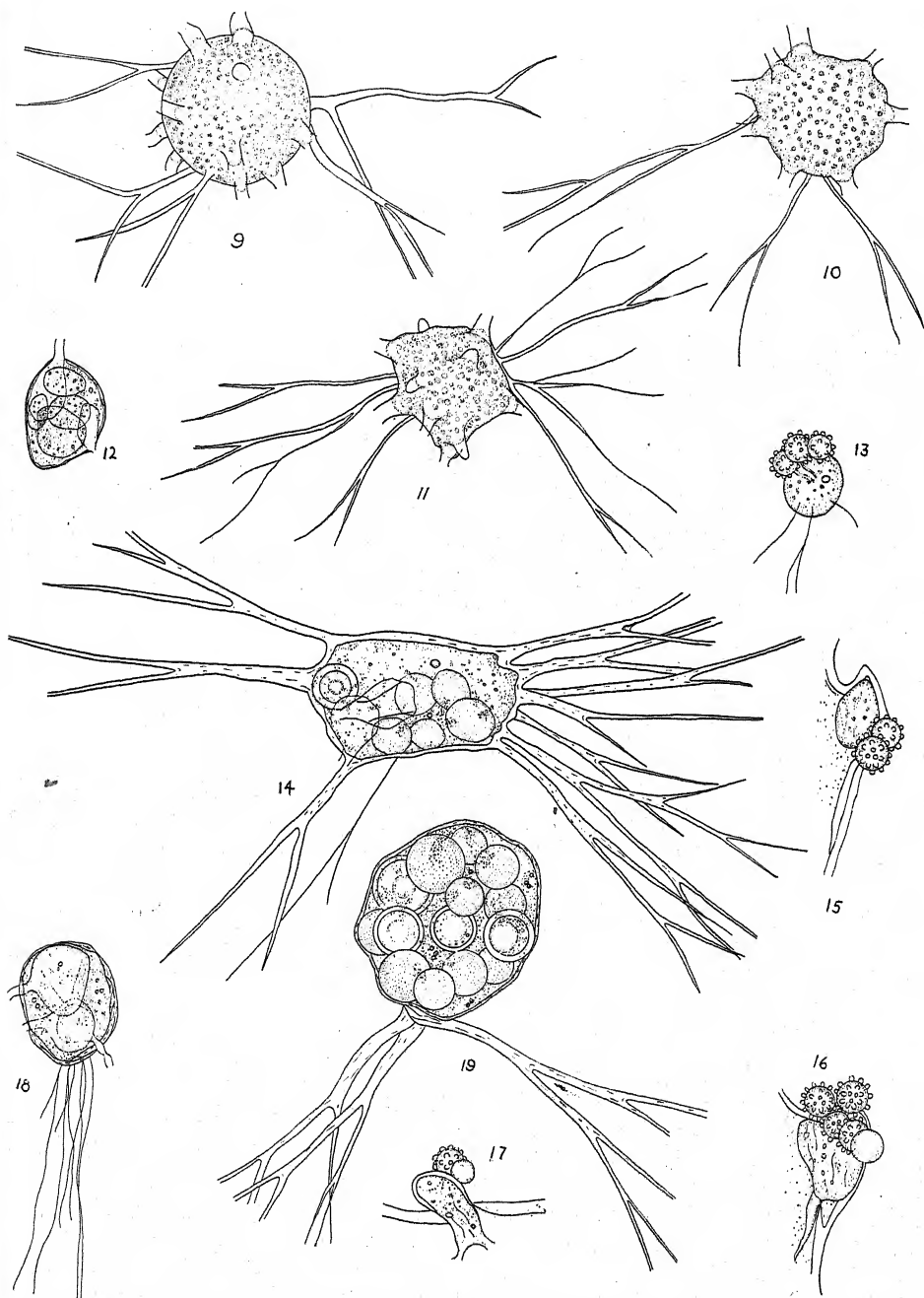
Parasitic in thalli, sporangia and resting spores of *Rhizophlyctis* spp., Bikini, Eniwetok and Rongelap atolls, Marshall Islands.

In the thalli, sporangia and resting spores of the amber colored types of *Rhizophlyctis* from Bikini I., Enyu I., and Eniiruku I. of Bikini Atoll, Bogon I. of Eniwetok Atoll and Rongelap I. of Rongelap Atoll, there were found thalli of the endobiotic parasite (FIGS. 7, 8, 12, 14, 18, 19) described above. One to many spherical or ellipsoidal hyaline thalli $12-45 \times 25-50\mu$ were present in a single host plant. At maturity some of the parasitic thalli became converted into sporangia, each provided with a single slender discharge tube which penetrated the wall of the host and through which the zoospores were discharged. The zoospores were spherical, about 2μ in diameter, posteriorly uniflagellate and without the usual distinctive globule. Their movement was of a rapid and erratic hopping type. Other thalli became converted, apparently asexually, into resting spores (FIG. 7, 19). These were spherical, faintly brown, $12-20\mu$ in diameter with a smooth wall about 2μ in thickness. As many as sixteen have been found in a single host thallus. Their germination was not observed.

So far as the writer is aware, this is the first undoubted species of *Olpidium* parasitic on another Phycomycete and, with *O. Uredinis*, one of the only two species known to attack fungi. Like other members of the genus, it has little to distinguish it morphologically and is segregated on the basis of its host plant.

Rhizophidium marshallense n. sp.

Sporangium sessile, globosum, $10-12\mu$ diametro, hyalinum, leviter circumvallata; parte endobiotica ex rhizoideo tenui 1-ramoso constante; zoosporis subovoideis, ca. 2μ diametro, globulo singulo minuto refractivo et flagello posteriori praeditis, per poro singulo varie locato ad-exteriorem natantibus; sporis perdurantibus globosis, $10-15\mu$ diametro, pallide aureis, dense bullationibus prominentibus vestitis; parte endobiotica eae sporangii simili. Germinatio ignota.



FIGS. 9-19. Phycomycetes from Marshall Islands.

Parasiticum in thallis sporangiisque generis *Rhizophlyctidis* ad insulas Eniwetok et Rongelap, in Insulis Marshallianis.

Sporangium sessile, spherical, 10–12 μ in diameter, colorless, smooth-walled; endobiotic part consisting of a slender, at least once-branched, rhizoid; zoospores somewhat ovoid, 2 μ or less in diameter, each with a single hyaline, minute refractive globule and a posterior flagellum, escaping through a minute variously placed pore; resting spore spherical, 10–15 μ in diameter, faintly golden and densely covered with prominent knoblike bullations, endobiotic part like that of the sporangium; germination not observed.

Parasitic on thalli and sporangia of *Rhizophlyctis* spp., Eleuge-lab I. Eniwetok Atoll and Rongelap I., Rongelap Atoll, Marshall Islands.

This species of chytrid was found attacking thalli and sporangia of the rose colored and amber colored forms of *Rhizophlyctis*. Mature thalli consisted of a spherical, 10–12 μ in diameter, smooth-walled, colorless, epibiotic part sessile on the wall of the host, and a very slender, at least once-branched, endobiotic rhizoidal system. The zoospores formed by the sporangia were 2 μ or less in diameter, posteriorly uniflagellate, and escaped through one variously located, minute pore. Resting spores were produced quickly and in great abundance. They were apparently asexually formed from thalli similar to those which gave rise to zoosporangia. They were spherical, 10–15 μ in diameter, faintly golden colored and densely covered with prominent hyaline bullations. Their germination was not observed.

A comparison of this chytrid with congeneric parasitic forms possessing resting spores with ornamented walls reveals that by reason of its small size, spherical uniporous sporangia and host it is a distinct, hitherto undescribed species.

EXPLANATION OF FIGURES

All figures $\times 330$. FIGS. 1–3. Large amber colored sporangia of *Rhizophlyctis* spp. FIGS. 4, 8. Sporangia characteristic of the smaller size class of amber colored forms. The sporangium of figure 8 bears within it the thalli of an endobiotic parasite. FIGS. 5, 6. Brown resting spores associated with populations of amber colored forms. FIG. 7. Thallus bearing the resting spore of an *Olpidium* within it. FIGS. 9–11. Sporangia of *Rhizophlyctis* illustrative of populations of pink to red colored forms. FIG. 12. Thallus of *Rhizophlyctis* heavily infected with *Olpidium Rhizophlyctidis* n. sp. FIG. 13. Thallus of *Rhizophlyctis* bearing resting spores of *Rhizo-*

phidium marshallense n. sp. FIG. 14. Peltate thallus of *Rhizophlyctis* with thalli, sporangia and one resting spore of *Olpidium Rhizophlyctidis*. FIGS. 15-17. Sporangia and resting spores of *Rhizophidium marshallense* on thalli of red pigmented forms of *Rhizophlyctis*. FIGS. 18, 19. Thalli, empty sporangia and resting spores of *Olpidium Rhizophlyctidis* on amber colored forms of *Rhizophlyctis*.

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NOTES ON THE GENUS *CYSTODERMA*

ALEXANDER H. SMITH AND ROLF SINGER

(WITH 1 FIGURE)

Since publishing our monograph on the genus *Cystoderma*¹ further information worthy of note has accumulated. A new species with very peculiar characters was discovered on the slopes of Mt. Hood, Oregon. A second undescribed species was found south of Mt. Hood, and a number of other interesting collections have been made in the Mt. Hood National Forest.

SUBGENUS *Dissoderma* subg. nov.

A subgenere *Eu-Cystoderma* subgen. nov. (= *Cystoderma* in limitibus Monographiae auctorum) differt epithelio summopere heterogeneo, in pileo evolutione in juvenilibus terminato et expansione pilei dissociato delapsoque ita ut pilei maturi epicute careant; sporis inamyloideis, majusculis.

Cystoderma paradoxum sp. nov. (FIG. 1).

Pileo 1-3 cm. lato, convexo, appresse fibrilloso, pallide lilaceo vel obscure violaceo-livido, pallidiore in speciminibus siccioribus, in primordiis velo granuloso ferrugineo celluloso oblecto sed dein denudato tegumenti separatione a superficie pilei. Lamellis distantibus vel subdistantibus, latis, arcuato-adnatis vel subdecurrentibus, interdum postremum adnexis et dente decurrentibus et tunc frequenter ventricosis in maturis, pallide brunneo-lividis vel pallidissime opace purpurascentibus, aciebus integris. Stipite 4-8 cm. longo, 4-6 mm. lato in tertia apicali, 6-8 mm. deorsum, pallide lilaceo lacero-squamoso longitudinaliterque striato in tertia apicali, solido, in duobus tertiis inferioribus cothurnato tegumento velari granuloso, cinnamomeo-alutaceo vel argillaceo-brunneo quo rupto armillis granulosis concentricis vel fasciculis vel squamis oblectae apparent. Carne grisello-violacea, violacea vel lilacea in pileo et in tertia apicali stipitis, argillaceo-brunneo in parte inferiore stipitis; odore saporeque haud notabilibus. Sporis plerumque 9-10 \times 4.5-6 μ , ellipsoideis, laevibus, membrana simplici, continua, hyalina vel subhyalina (in KOH), subpseudoamyloideis, haud amyloideis; basidiis 30-35 \times 7.5-10 μ ; cystidiis nullis; tramate hyalino; cuticula pilei (primordiis exceptis) reducta, ex zona densiuscula et pigmentata consistente, hyphis filamen-

¹ A monograph on the genus *Cystoderma*. Papers Mich. Acad. Sci. Arts and Letters 30: 71-124. 1945.

tosis efformata; fibulis numerosis; tegumento stipitis granuloso e catenulis cellularum ovoidearum, ellipsoidearum vel globosarum (flavido-brunnearum vel ferrugineo-vinacearum in KOH) consistente.

Ad humum et inter Bryophyta, solitarius vel e massa carnosu basali ecrescens. Octobri. Legit A. H. Smith in Mt. Hood National Forest, Oregon, Americae Borealis. **Typus** (n. S-27987) in Herbario Universitatis Michiganensis conservatus est.

Pileus 1-3 cm. broad, convex to broadly convex, sometimes with a slightly depressed disc and sometimes with a low broad umbo,

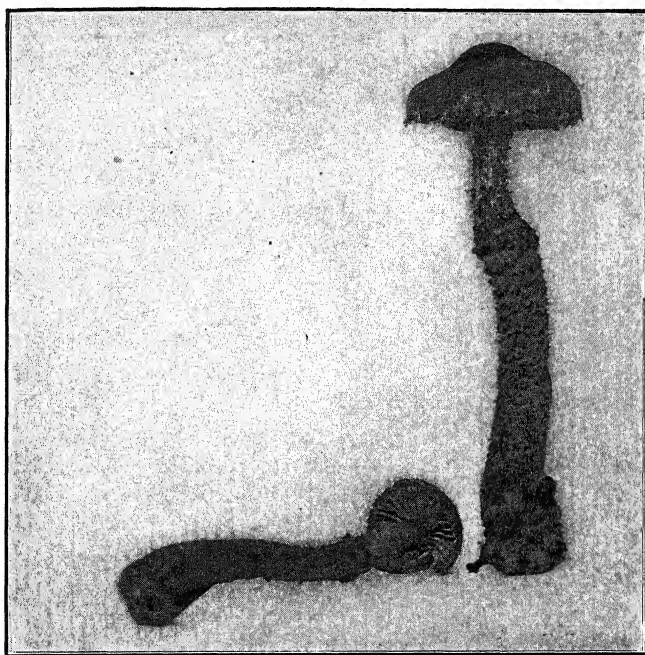


FIG. 1. *Cystoderma paradoxum*.

surface dry and appressed fibrillose, sometimes the fibrils aggregated into fascicles near the margin, matted down like felt over the disc, color pallid lilac to dark violaceous drab ("dusky drab" to "dusky brown" on disc, margin "pale brownish drab") in wet caps, paler lilac when not water soaked, some when badly water soaked translucent-striate on margin and appearing atomate when dried out at room temperature; buttons covered by a "Sudan brown" or duller granulose veil which is only loosely connected to tissue of pileus, the granules (sphaerocysts) resembling those found in *C. amianthinum* f. *typicum*, granulose coating separating from

pileus surface and sloughing off in early stages of pileus expansion thus exposing the lilaceous fibrillose cuticle as observed on mature caps; flesh thin, soft, watery, "pale brownish drab" to grayish violaceous, odor and taste not distinctive; lamellae distant to subdistant, broad, variable in manner of attachment, arcuate-adnate to subdecurrent or in some bluntly adnate becoming adnexed, with a slight tooth and this type sometimes becoming ventricose, "pallid brownish drab" to "pale ecru drab" (very pale dull purplish), edges even; stipe 4-8 cm. long, 5-6 mm. in apical third, 6-8 mm. in lower two thirds, upper third pale lilac or violaceous, and lacerate scaly as well as longitudinally striate, lower two thirds sheathed by a granulose "cinnamon buff" to "clay color" veil which is broken up into more or less concentric rings of tufts or scales, flesh within clay color in lower two thirds, lilac to violaceous in upper third, solid.

Spores $(8.5)9-10(11) \times 4.5-6 \mu$, ellipsoid, smooth, the wall thin to very slightly thickened, simple, hyaline or subhyaline in KOH and NH_4OH , yellowish to pale tawny (hence slightly pseudoamyloid) in iodine (most coloration obtained when plunged in alcohol, then in water, and finally stained with Melzer's reagent; least coloration obtained with ammonia followed by Melzer's reagent); basidia four-spored, $30-35 \times 7.5-10 \mu$, clavate, hyaline in KOH; pleurocystidia and cheilocystidia not differentiated; gill trama hyaline in KOH, its hyphae interwoven—subparallel (not distinctly irregular); pileus trama hyaline in KOH or near surface faintly brownish gray; cuticle differentiated only as a slightly denser and pigmented zone, its elements of various shapes and without orderly arrangement, filamentous elements predominantly repent, thickened elements predominantly vertically arranged, both kinds often interwoven and with walls only slightly thicker than in trama proper; clamp connections present; covering of stipe (lower two-thirds) consisting of chains of oval, ellipsoid or globose cells with yellowish brown to rusty vinaceous walls in KOH, the walls slightly thickened, some cells rather irregular in outline or with one or two papillae; cells on surface of buttons rusty to cinnamon in KOH, mostly globose to short ellipsoid.

Habit, habitat, and distribution. Solitary or in small clusters from a fleshy basal mass of tissue. On humus and among mosses. Mt. Hood National Forest, Oregon. The following collections have been made: S-24843, Oct. 21, 1946; S-25003, Oct. 26, 1946; S-27824, Oct. 16, 1947; S-27987, Oct. 19, 1947; and S-28341, Oct. 27, 1947. The specimens illustrated, S-27987, are designated the type.

Discussion. We withheld publication of this species until we could ascertain the type of cuticle present in button stages. Small buttons were collected in S-27824 and the cuticle found to be similar to that of the typical form of *C. amianthinum*. The peculiar features of this fungus are many. In the first place the manner in which the cellular covering is sloughed off, so completely that no remains are left on the cap, is a sufficiently important character to justify erecting a subgenus for the species. The peculiar color of the pileus, gills and upper third of the stipe is very distinctive as a field character.

There are still some questions to be answered in regard to this fungus. One is whether or not it really grows from very decayed mushrooms or is truly terrestrial. S-25003 gave some evidence of having come from a decayed fruiting body of a fleshy fungus but the remains were too far gone for any identification. No evidence was found during 1947 to suggest such a substratum, but again, all collections had either matured fruiting bodies or buttons which had ceased developing. Stages in the development of the buttons are greatly desired in order to learn more about the manner of disjunction of the covering layer of the pileus.

Cystoderma subpurpureum sp. nov.

Pileo 4-8 mm. lato, convexo, expanso vel plano, sicco pulverulentoque atque obscure livido-brunneo, maturando pallide grisello-vinaceo, demum subdiscolorato. Lamellis late adnatis, confertis vel subdistantibus, latis, dilute rubido-vinaceis vel brunneolo-vinaceis, aciebus integris. Stipite 1-2.5 cm. longo, usque ad 1 mm. lato, pileo concolori, exsiccando purpurascens, fragmentis sparsis veli pulverulenti oblecto, zona apicali fibrillosa aut annulo mox evanescente instructo, apice pruinoso. Carne pilei vinacea, tenui; odore nullo; sapore miti. Spor. $4-4.5 \times 2.5-2.8 \mu$, ovoideis, laevibus, hyalinis, inamyloideis; basidiis $18-20 \times 4.5-5 \mu$; cystidiis nullis; tramate hymenophorali in juvenilibus subviolaceo-grisello (in KOH), dein subhyalino; tramate pilei subviolaceo-griseo vel hyalino in KOH; epithelio pilei primum obscure violaceo-livido in KOH, gradatim autem depallentibus usque ad sordide brunneolo-violaceum; fibulis praesentibus.

Ad locum deustum Mt. Hood National Forest, Oregon Americae Borealis Septembri 1947. Legit A. H. Smith (n. S-26824), *typus*, in Herbario Universitatis Michiganensis conservatus est.

Pileus 4-8 mm. broad, convex, expanding to plane or the margin arched and disc slightly depressed; surface dry and powdery at first, "deep livid brown" young, "pale grayish vinaceous" near ma-

turity, in age when faded ashy with a purple-drab cast (dark haematite color to pale ashy pink and fading to ashy in age); flesh thin, tinged vinaceous, odor and taste none, lamellae broadly adnate, close to subdistant, broad, "light russet-vinaceous" to "deep brownish vinaceous" (pale haematite color), edges even; stipe 1–2.5 cm. long, 1 mm. or less thick, concolorous with pileus and drying more purplish, with scattered remains of the thin powdery veil and with a fibrillose apical zone or annulus which is soon evanescent, apex pruinose.

Spores $4-4.5 \times 2.5-2.8 \mu$, ovoid, smooth, hyaline, nonamyloid; basidia four-spored, $18-20 \times 4.5-5 \mu$; pleurocystidia and cheilocystidia none; gill trama in young caps with a violaceous gray cast in KOH, in mature caps nearly hyaline, parallel becoming subinterwoven; pileus trama violaceous gray to hyaline in KOH; cuticle of globose to short-ellipsoid cells, or a few scattered fusoid cells also present, dark violaceous drab at first when mounted in KOH, gradually clearing to a dull brownish violaceous, the cell walls smooth and slightly thickened; clamp connections present.

Habit, habitat, and distribution. Scattered over an area where a brush pile had been burned, Clear Creek at Skyline Rd., Mt. Hood National Forest, Oregon. September 25, 1947 (S-26824 type).

Discussion. This fungus differs from the accounts of *C. haematites* by English authors in the much darker gills even if one wishes to disregard the striking difference in size. *C. haematites*, according to Kühner and Maire,² has amyloid spores, and this, of course, places the two species in different sections. In our system of classification *C. subpurpureum* would be in our section *Granulosa*. It differs from *C. granulorum* var. *occidentale* in the KOH reaction of the sphaerocysts as well as in its darker colors and slender stature.

CYSTODERMA GRUBERIANUM Smith³

This interesting species with large ($9-11.5 \times 5-6 \mu$), subfusiform, amyloid spores belonging in the *C. amianthinum* series

² Étude de la réaction de la membrane sporique à l'iode dans les divers genres d'Agarics leucosporés. Bull. Soc. Myc. Fr. 50: 9-24. 1934.

³ Mushrooms in their natural habitats illustrated with stereokodachromes. Sawyer's Inc., Portland, Oregon. (In press.)

should be called to the attention of those interested in this genus. It is a lignicolous species inhabiting rotting Douglas fir logs. A second collection (S-28239) has been obtained from the type locality. In it the spores measure up to $12.5\ \mu$ long and are strongly amyloid.

SUPPLEMENTARY NOTES ON KNOWN SPECIES

C. GRANULOSUM (Batsch ex Fr.) Fayod var. *TYPICUM* f. *TYPICUM*. Material from Errol, N. H. (Linder & Rusden, det. Singer Sept. 17, 1945, FH) and Hamilton, old Harvard Forest, Mass. (Singer, Oct. 24, 1945, FH) shows that the type form is not rare in New England.

C. GRANULOSUM var. *ADNATIFOLIUM* (Peck) Smith & Sing. Material from Mt. Monadnock, N. H. (Linder & Rusden, det. Singer, Sept. 20, 1945, FH) is the first material studied by one of us coming from a New England locality. Although this extension may have been expected, as the variety was known from both New York and Eastern Canada, we think it worthy of record.

C. CINNABARINUM (A. & S. ex Secr.) Fayod. A collection from Harvard, Mass. (Singer & Dadmun, September 1944, FH) is the first indubitably correct indication from Massachusetts. More important is a specimen received by the Farlow Herbarium in exchange from France (Lacorube, région Lyonnaise, coll. Locquin, no. 160.44, October 16, 1944). It has very distinct cystidia with crystals at the tip, and is entirely identical with our American material as well as with the junior author's Siberian material. Locquin is a French specialist of *Lepiota* sensu lato and it is significant that his determination (which reads: *Lepiota cinnabarina*, sensu Kühner, Locquin) fully coincides with ours.

C. CARCHARIAS f. *ALBUM* (Fr.) Smith & Sing. This form, previously reported for North America only from one collection in Michigan, was found near Rhododendron, Oregon, Oct. 27 (S-28882), and near Sandy, Oregon, Nov. 6, 1947 (S-28527). Collection S-28332 was from a moss carpet under second growth Douglas fir, and S-28527 was from a very dense growth of *Rubus*.

alder and maple, the typical "jungle" growth of the Humid Transition zone.

C. AMIANTHINUM var. *SUBLONGISPORUM* Sing. apud Smith & Sing. A collection from Virginia (Singer, White Top Mountain, no. V193, summer 1946, FH) extends the area of this variety to Virginia.

THE DISTRIBUTION OF SOIL MICRO-ORGANISMS ANTAGONISTIC TO FUNGI PATHOGENIC FOR MAN *

ALBERT SCHATZ † AND ELIZABETH L. HAZEN

(WITH 3 FIGURES)

Although the majority of investigations on antagonistic relationships among microorganisms have been primarily concerned with inhibition of bacteria, there are numerous reports on the anti-fungal properties of various microorganisms and cell-free preparations obtained from them. No attempt will be made to discuss this extensive literature reviewed so well by Waksman (1). These studies dealt chiefly with the antagonism of saprophytic or plant-pathogenic fungi and the fungistatic or fungicidal properties of more or less nonspecific substances produced by active cultures. Only a few (2-10) are comparable in scope to surveys on the distribution of microorganisms antagonistic to bacteria (11-15). No surveys conducted to investigate microorganisms antagonistic to fungi pathogenic for man have to our knowledge been reported.

A variety of fungi pathogenic for man, such as *Trichophyton schoenleini*, *Blastomyces dermatitidis*, *Candida albicans*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Microsporium gypseum*, *Trichophyton gypseum* and *Trichophyton mentagrophytes*, as well as plant pathogens and nonparasitic fungi, have been tested for susceptibility *in vitro* to different antibiotic substances. The list of agents includes actinomycin (16, 17), chaetomin (17), clavacin (16, 17, 18), eumycin (19), fumigacin (16, 17), gladiolic acid (20), gliotoxin (16, 17, 21, 22), glutinosin (23), hemipyocyanine (24), mycophenolic acid (25), penicillin

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(16, 26, 27), pyocyanase (24), simplexin (28), streptomycin (17, 29), streptothricin (17), tyrothricin (24), viridin (30), and others.

The majority of these substances were found to be highly inhibitive to fungi; eumycin, gladiolic acid, glutinosin, and viridin being appreciably more active against fungi than bacteria. On the other hand, certain antibiotics such as chaetomin, penicillin, and streptomycin exerted little or no effect on the microorganisms tested. Unfortunately, the preparations that appeared most promising *in vitro* have no widespread application because of toxicity or other undesirable pharmacologic properties. Consequently, no antibiotic agent approaching the efficacy of penicillin and streptomycin against bacterial infections is available in fungus infections either of the superficial or the deep-seated type. For this reason, experiments were undertaken to investigate (1) the distribution of soil microorganisms antagonistic to fungi pathogenic for man and (2) the antifungal agents produced by these microorganisms. This report presents chiefly the results of the study of the distribution of soil microorganisms antagonistic to certain pathogenic fungi employed as test microorganisms.

MATERIALS AND METHODS

Several different substrates were chosen as sources of microflora. Almost all the studies, however, were made with a deciduous forest soil, a field soil, and a 2 to 3-year-old leaf compost, the pH values of which were approximately 5.3, 8.4, and 6.1, respectively. A peat and a garden soil both at pH 4.5 were considered unsatisfactory and discarded after preliminary experiments because of the generally low microbial population and the almost complete absence of actinomycetes.

Three tap water media used in previous studies for the isolation of microorganisms antagonistic to bacteriophages (31, 32) were employed: nutrient broth (NaCl 0.5 per cent, peptone 0.5 per cent, meat extract 0.3 per cent); glucose-tryptone broth (glucose 1 per cent, tryptone 0.5 per cent, K_2HPO_4 0.2 per cent, NaCl 0.2 per cent, $FeSO_4$ 0.001 per cent); glycerol-yeast extract broth (glycerol 3.15 per cent, yeast extract 0.3 per cent, NaCl 0.2 per cent, K_2HPO_4

0.1 per cent, MgSO_4 0.02 per cent, FeSO_4 0.001 per cent). For liquid cultures of the actinomycetes, 0.15 per cent agar was added to the broths to obtain sufficient viscosity for supporting pellicles, and for the solid media, 1.5 per cent agar was added. All the media were adjusted to pH 6.8–7.2. A neutral tap water dextrose-asparagin agar (dextrose 1 per cent, asparagin 0.05 per cent, K_2HPO_4 0.05 per cent, agar 1.5 per cent) was also employed for some of the studies. The media containing glucose were autoclaved at eight pounds pressure for thirty-five minutes; the others were sterilized at fifteen pounds pressure for twenty minutes.

The pathogenic fungi employed as test microorganisms were *Candida albicans* (No. 4657), *Cryptococcus neoformans* (No. 45215), *Trichophyton gypseum* (No. 45141), and *Trichophyton rubrum* (*purpureum*) (No. 4516). The cultures were maintained on slants of glucose-tryptone agar and suspensions were prepared by adding 5 ml. of sterile saline or broth to an agar slant and scraping the growth from the surface with a stiff wire loop.

The soil microorganisms were isolated at random and also by selective plating technics. One method employed was a modification of Foster and Woodruff's procedure (33). The soils and compost were plated in agar seeded with a slow-growing fungus. After three to four days, the colonies of the soil microorganisms that had developed rapidly and produced diffusible antifungal substances were surrounded by clear zones in which the pathogen had been inhibited. From the clear zones the antagonists were transferred to agar slants. In addition, the soils and compost were plated and incubated for from five to six days. A second layer of fungus-seeded glucose-tryptone agar was then poured over the surface of those plates with well-isolated colonies, care being taken to prevent bacterial colonies from flowing and contaminating the entire surface. After from one to four days of further incubation, antagonistic microorganisms in the original layer of agar that were surrounded by clear zones of inhibition of the pathogenic fungus in the second agar layer were removed.

The procedures for the agar-streak, agar-dilution, and agar-cup or diffusion tests employed were essentially the same as those previously described for studies with fungi (17). The three agar media were used for the agar-streak tests, and glucose-tryptone

agar was used throughout for the dilution and diffusion assays. All incubation was at approximately 28° C., the duration varying with the particular experiment and the rate of growth of the test microorganisms.

EXPERIMENTAL

It was assumed *a priori* that the actinomycetes would constitute the most fruitful group among which to search for antifungal properties. The better known antibiotics of bacterial origin, such as tyrocidine, tyrothricin, subtilin, bacillin, bacitracin, simplexin, are more or less similar in chemical nature and antibacterial activity which is largely against Gram-positive bacteria. The agents produced by fungi and actinomycetes, on the other hand, appear to represent more heterogeneous types of compounds chemically, pharmacologically, and antibiotically. In numerous agar platings of mixed microbial populations from such natural substrates as soils, manures, and composts, the antagonism of fungi by actinomycetes rather than by molds has been more frequently observed. This phenomenon is illustrated by figure 1 in which all seven colonies antagonizing the spreading fungus are actinomycetes. For these reasons, attention was directed to the potentialities of the actinomycetes.

Two hundred and forty-three actinomycetes, one hundred and ninety-eight isolated at random from a leaf compost, a peat, and five soils, and forty-five previously reported to be antagonistic to bacteriophages (32) were tested on the three agar media by the streak method against the four pathogenic fungi. The actinomycetes were incubated for two days before being cross-streaked with the four pathogens. Because of poor growth, *Cryptococcus neoformans* was not tested on the nutrient agar. As illustrated in figure 2, antagonistic actinomycetes exhibited zones of inhibition from two to three days after the fungi were inoculated.

Those actinomycetes that were appreciably active by the agar-streak procedure were grown in liquid media, 100 ml. per 500-ml.-Erlenmeyer flasks. After incubation for ten days, the cultures were filtered through sterile paper. Untreated samples of the filtrates were assayed by the agar-diffusion or cup technic against

Candida albicans and *Cryptococcus neoformans*. Because of the rapid growth of the former, zones of inhibition were measured after approximately eight hours. The plates with *Cryptococcus neoformans*, however, were incubated overnight (20–24 hours) before being read. Portions of a large number of the filtrates were heated

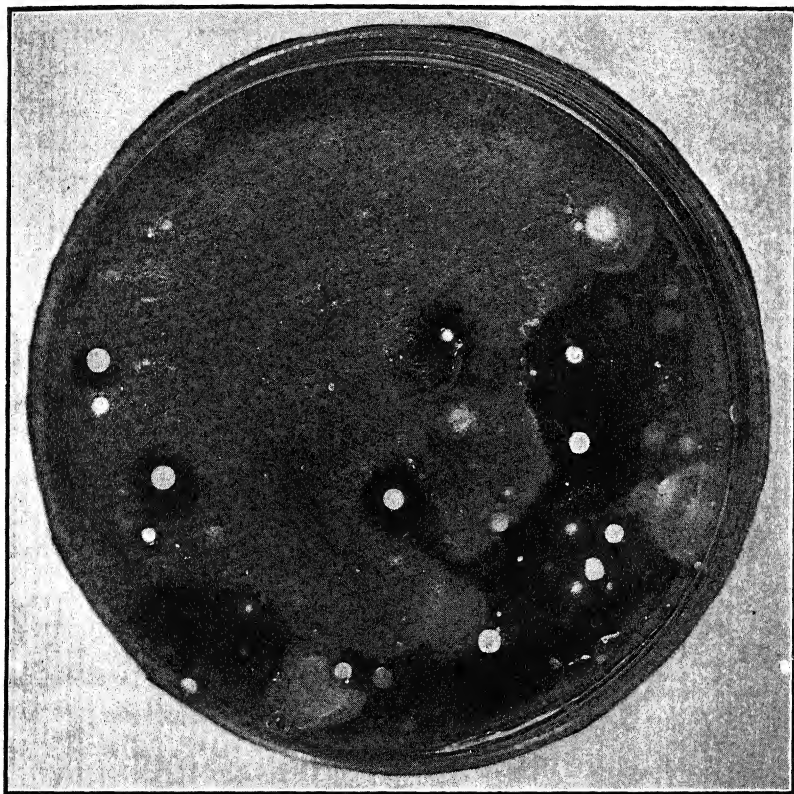


FIG. 1. Antagonism among soil microorganisms. The inhibition of a spreading fungus by actinomycetes.

at 70–75° C. for ten minutes and tested by the agar-dilution method against all four pathogens.

Table I shows that one hundred and twenty-four (51 per cent) of the two hundred and forty-three actinomycetes tested by the agar-streak method were active against one or more of the test microorganisms on at least one of the three media employed. This

value is of the same order of magnitude as the percentage of actinomycetes reported to be antagonistic to bacteria, namely 59 per cent by Nakhimovskaia (14) and 43 per cent by Waksman *et al.* (11). When the heated culture filtrates were tested by the agar dilution and diffusion procedures, however, only 10 per cent and

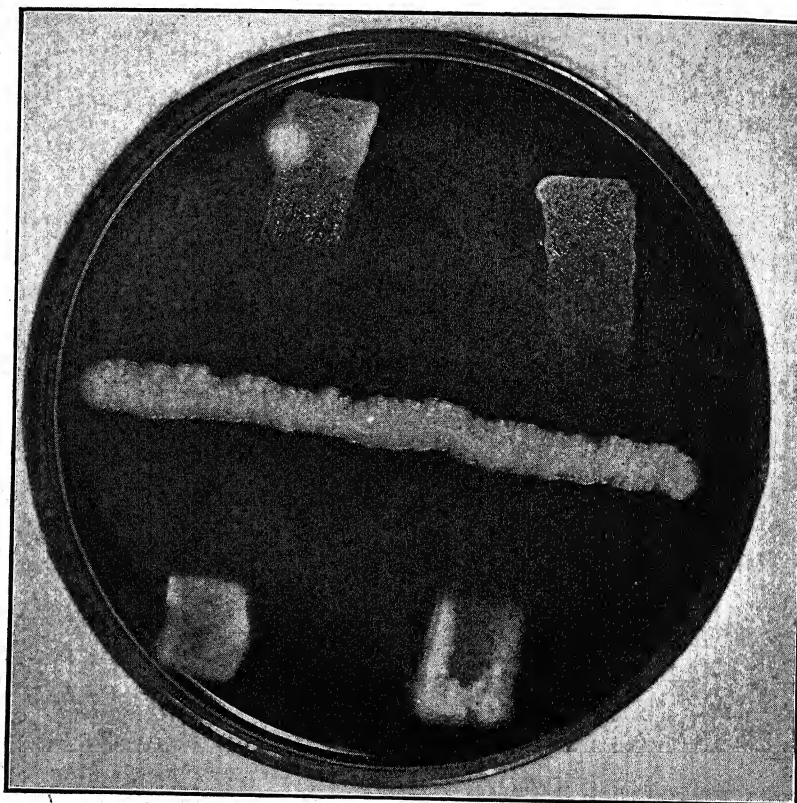


FIG. 2. Streak test showing antagonism of four pathogenic fungi by a soil actinomycetes.

43 per cent, respectively, exhibited antifungal properties. The fact that approximately four times as many filtrates were active by the diffusion method as by the dilution technic would appear to indicate that a rather high percentage of the filtrates contained heat-labile agents which were destroyed in the heating process. That this was not true was demonstrated by simultaneously cup-assaying

TABLE I
THE DISTRIBUTION OF ANTIFUNGAL PROPERTIES AMONG SOIL ACTINOMYCETES ISOLATED AT RANDOM

Source of Cultures	Agar-Streak Test				Agar-Dilution Test				Agar-Diffusion Test						
	Total Active Cul- tures*	Antagonists against†			Total Active Cul- tures	Filtrates			Total Active Cul- tures	Filtrates					
		CA	CN	TG		TP	Total Num- ber Active	Inhibition of							
								CA		CN	TG	TP			
Forest soil	19/38	15	18	18	17	4/19	4/47	0	4	1	2	22/47	3	11	8
Leaf compost	12/25	10	6	6	11	0/10	0/16	0	0	0	0	4/10	1	3	0
Cornfield soil	18/36	14	15	10	8	1/11	2/23	0	2	0	0	4/10	6/20	3	1
Peat	10/18	9	8	8	8	2/8	3/20	0	3	1	1	5/8	11/21	3	4
Field soil	28/53	20	18	20	22	3/17	7/35	4	7	6	6	10/18	19/36	4	7
Miscellaneous garden soils	13/28	10	9	10	11	0/4	0/12	0	0	0	0	4/7	4/15	1	3
Aniphage actinomycetes	24/45	17	4	7	13							0/1	0/0	0	0
Total	124/243	95	78	79	90	10/69	16/153	4	16	8	9	40/73	66/155	15	30
Per cent	51					15	10					55	43		21

* Numerator = number of cultures or filtrates active on one or more media against at least one fungus; denominator = total number of cultures or filtrates tested.

† CA = *Candida albicans*; CN = *Cryptococcus neoformans*; TG = *Trichophyton gypseum*; TP = *Trichophyton rubrum* (*purpureum*).

heated and unheated samples of twenty filtrates previously shown to be active by the diffusion but not by the dilution method. The results of these tests, in which the preparations proved to be heat-stabile, indicated that the cup assay was more sensitive than the dilution method.

The influence of composition of the media on the antagonism of fungi by soil actinomycetes is shown in Table II. In general, it

TABLE II
THE INFLUENCE OF COMPOSITION OF MEDIA ON THE DISTRIBUTION OF
ACTINOMYCETES ANTAGONISTIC TO PATHOGENIC FUNGI *

Source of Cultures	Agar-Streak Test															
	Nutrient Agar Antagonists against†			Glucose-tryptone Agar Antagonists against				Glycerol-yeast Extract Agar Antagonists against				Percentage of Total Streaks Active against‡				
	CA	TG	TP	CA	CN	TG	TP	CA	CN	TG	TP	CA	CN	TG	TP	
Forest soil	14	16	15	14	16	13	14	10	14	13	12	33	40	36	36	
Leaf compost	8	3	7	1	2	3	4	4	6	4	5	20	16	15	25	
Cornfield soil	8	6	7	7	9	6	5	8	14	4	5	21	32	15	16	
Peat	6	5	4	3	5	6	6	6	6	7	7	28	31	33	32	
Field soil	13	11	11	12	15	18	17	15	16	17	18	25	29	29	28	
Miscellaneous garden soils	7	8	8	6	10	11	12	7	7	7	9	24	20	30	34	
Antiphage actino- mycetes	15	0	3	1	1	1	7	2	3	7	10	13	4	6	15	
Total	71	49	55	44	58	58	65	52	66	59	66					
Per cent	29	20	22	18	24	24	27	21	27	24	27					

* See Table I for total number of cultures tested from each source.

† CA = *Candida albicans*; CN = *Cryptococcus neoformans*; TG = *Trichophyton gypseum*; TP = *Trichophyton rubrum* (*purpureum*).

‡ $\frac{(\text{Total antagonists (Table II)}) (100)}{(\text{Total cultures tested (Table I)}) \times (\text{Number of test media})} = \text{Per cent of total streaks active.}$

appears that each fungus was antagonized by more or less the same percentage of actinomycetes on all three media. The results in Table II indicate that for these four fungi, at least, there is no great variation in sensitivity which approaches the difference between Gram-positive vis-à-vis Gram-negative bacteria (11, 15). Actually, there were some streak tests in which the actinomycetes exhibited activity against two or three of the four test fungi; only an occasional microorganism was active against but one of the

pathogens. On the other hand, the tests with culture filtrates (Table I) showed that *Cryptococcus neoformans* was in general the most sensitive of the four fungi; *Candida albicans* was the most resistant; the two species of *Trichophyton* were intermediate and both of the same order of sensitivity. This variation in susceptibility to culture filtrates is similar to that obtained when such preparations are tested against bacteria.

Table III presents the results of a more detailed study of broth filtrates of four of the antagonistic actinomycetes. The dependence of the production of antifungal agents on composition of the medium, the rate of production of the active substances, and the selective nature of the inhibitive effect are indicated here. The nutrient broth was apparently a much poorer substrate than the glucose-tryptone or glycerol-yeast extract media. It is of interest to mention here that none of these culture filtrates exhibited any activity against *Bacillus subtilis*, *Escherichia coli*, or *Mycobacterium phlei* by the agar-dilution method.

From the antifungal spectra of the culture filtrates (Table III), it appears that cultures No. 47204 and No. 47205 may produce the same or similar agents, but that microorganism No. 4779 is decidedly different, especially with respect to activity against *Candida albicans* and lack of inhibition of the trichophyta by the agar-dilution test.

Table II shows that the forest soil contained a somewhat higher percentage of antagonistic actinomycetes than did the field soil, peat, leaf compost, cornfield soil, or miscellaneous garden soils. The actinomycetes that had been found to be antagonistic to bacteriophages were the least active group against the pathogenic fungi. Approximately the same order of activity was obtained when these cultures were tested against nonparasitic fungi. From data not reported here, only eight of fifty-four antiphage actinomycetes were antagonistic on one or more media to a *Trichoderma* sp. and a strain of *Aspergillus* which appeared to be *Aspergillus unguis*. In contrast to this, thirty-five of the actinomycetes antagonized *Streptomyces griseus*. The lower activity of the antiphage microorganisms may have been due to the fact that these cultures had been maintained on dextrose-asparagin agar for approximately one year, whereas the other microorganisms were fresh

TABLE III
ANTIFUNGAL ACTIVITY OF FOUR ANTAGONISTIC SOIL ACTINOMYCETES *

Culture No.	Broth Medium	Incubation Period, Days	Agar-dilution Test, Units per ml. against†				Agar-diffusion Test, mm. Zone of Inhibition against	
			CA	CN	TG	TP	CA	CN
47379	Nutrient broth	9	0	0	0	0	0	18
		13	0	0	0	0	0	20
		16	0	0	0	0	0	18
	Glucose-tryptone	9	0	75	0	0	0	>35
		13	0	50	0	0	10	>35
		16	0	5	0	0	0	20
	Glycerol-yeast extract	9	0	10	0	0	10	>35
		13	0	75	0	0	10	>35
		16	0	60	0	0	12	>35
47204	Nutrient broth	9	0	0	0	0	20	>35
		13	0	5	0	0	20	35
		16	0	0	0	0	16	32
	Glucose-tryptone	9	0	20	5	5	18	>35
		13	0	25	5	5	20	35
		16	0	25	5	10	18	32
	Glycerol-yeast extract	9	0	25	5	5	16	32
		13	0	15	0	0	16	32
		16	0	5	0	0	16	30
47205	Nutrient broth	9	0	5	0	5	12	30
		13	0	5	0	0	12	30
		16	0	5	0	0	18	32
	Glucose-tryptone	9	0	20	10	5	14	35
		13	0	75	5	10	18	35
		16	0	60	15	15	18	35
	Glycerol-yeast extract	9	0	15	0	0	18	30
		13	0	30	5	5	18	35
		16	0	25	0	5	18	35
4779	Glucose-tryptone	9	0	5	0	0	10	15
		13	0	10	0	0	12	18
		16	0	10	0	0	10	18
	Glycerol-yeast extract	9	10	30	0	0	16	22
		13	20	75	0	0	16	24
		16	10	75	0	0	16	24

* CA = *Candida albicans*; CN = *Cryptococcus neoformans*; TG = *Trichophyton gypseum*; TP = *Trichophyton rubrum* (purpureum).

† Number of units = reciprocal of the final dilution of culture filtrate per ml. of agar that just inhibits growth of the test microorganism.

isolates. It is well known that actinomycetes when transferred on synthetic media for long periods of time tend to lose certain properties.

In order to obtain information on the relative abundance of bacteria and fungi in soil antagonistic to pathogenic fungi, two

selective technics were employed. The first method required that the test culture grow appreciably more slowly than the majority of soil microorganisms. *Trichophyton rubrum* (*purpureum*) proved to be the only one of the four fungi satisfactory in this respect. Table IV presents the data obtained when the forest soil, leaf

TABLE IV
DISTRIBUTION OF SOIL MICROORGANISMS* ANTAGONISTIC TO
Trichophyton rubrum (*purpureum*)

Source of Culture	Microorganisms	Nutrient Agar	Glycerol-yeast Extract Agar	Dextrose-asparagin Agar
Forest soil	Actinomycetes	$\frac{4.3 \uparrow}{30}$	$\frac{2.5}{22.5}$	$\frac{2.5}{5.5}$
	Bacteria	$\frac{0.3 \uparrow}{20}$	$\frac{0.1}{64}$	$\frac{0}{10}$
	Fungi	$\frac{0}{0}$	$\frac{0}{2}$	$\frac{0}{4}$
Leaf compost	Actinomycetes	$\frac{2.5}{56}$	$\frac{0.6}{48}$	$\frac{1.8 \uparrow}{32}$
	Bacteria	$\frac{0.2 \uparrow}{\text{TN}}$	$\frac{0.6}{\text{TN}}$	$\frac{0.3 \uparrow}{162}$
	Fungi	$\frac{0}{0.5}$	$\frac{0}{2}$	$\frac{0.5 \uparrow}{1}$
Field soil	Actinomycetes	—	$\frac{1.8}{36}$	$\frac{7.8}{24.5}$
	Bacteria	—	$\frac{0}{31}$	$\frac{0}{33}$
	Fungi	—	$\frac{0}{1.5}$	$\frac{0}{3}$

* Counts $\times 10^6$ = numbers per gm. of moist soil. Numerator = antagonistic colonies. Denominator = total colonies.

† Values representing single plate counts.

‡ TN = colonies too numerous to count.

compost, and field soil were plated in the three agar media seeded with *Trichophyton rubrum* (*purpureum*). The total number of microorganisms in each soil was determined by simultaneously plating the same soil dilutions with fungus-free agar. Unless otherwise indicated, the values represent averages from three to six plates.

In another type of experiment, the soils and leaf compost were

plated and counts made after from five to six days' incubation, following which the plates were carefully flooded with fungus-seeded glucose-tryptone agar. In figure 3, which illustrates a modification of this technic, the secondary layer of agar had been seeded with both *Candida albicans* and *Cryptococcus neoformans*,

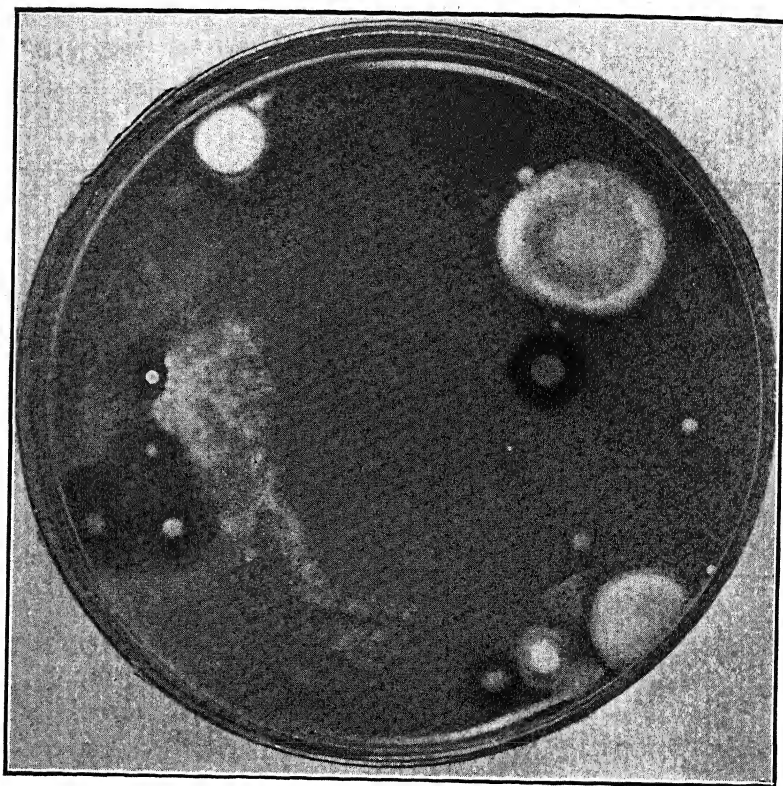


FIG. 3. Simultaneous antagonism of both *Candida albicans* and *Cryptococcus neoformans* by soil actinomycetes.

so that the zones surrounding six colonies of actinomycetes indicate simultaneous antagonism of both pathogenic fungi. The results obtained by this method with *Candida albicans*, *Cryptococcus neoformans*, and *Trichophyton gypsum* are presented in Table V. All values represent average counts from three to six plates selected because of well-isolated colonies and the absence of spreading

spore-formers at the time total counts were made and the second layer of seeded agar was poured.

It is evident from the data in Tables IV and V that the actinomycetes in general contain a much higher percentage of forms antagonistic to the pathogenic fungi than do the bacteria. Since fungi are considerably less numerous than actinomycetes and bac-

TABLE V

DISTRIBUTION OF SOIL MICROORGANISMS ANTAGONISTIC TO *Candida albicans*, *Cryptococcus neoformans*, AND *Trichophyton gypsum* *

Source of Culture	Microorganisms	Nutrient Agar			Glycerol-yeast Extract Agar			Dextrose-asparagin Agar		
		CA	CN	TG	CA	CN	TG	CA	CN	TG
Forest soil	Actinomycetes	8.5	9.5	4.4	8	15.3	10	0.8	2	0
		21.8	18.8	19	21	27.3	24.3	19.2	23.3	21
	Bacteria	0	0	0	0.3	0	0	0.2	0	0
		14.5	13.3	23.3	65.8	41	13.5	24	18.5	11.5
	Fungi	0.3	0	0	1.0	0	0	0	0	0.5
		1.5	0.5	0.8	3.8	1.5	2.5	4.0	4.3	5.8
Leaf compost	Actinomycetes	25	27	23	25	17	20	3.5	3	—
		40	73	37	30	47	32	8	16.5	—
	Bacteria	0	7	6	2	7	0	0	0	—
		345	660	1490	1240	1080	1360	385	416	—
	Fungi	0	0	0	2	0	2.5	0	0	—
		0	3	3	14	16	32.5	1.4	2.3	—
Field soil	Actinomycetes	6.5	4.8	3.5	4.8	8.5	5	2.3	3.0	2.8
		38	80.5	42.8	42.5	47.5	46.4	22.5	21.0	37.8
	Bacteria	0.5	0.5	0.5	0.3	0	0	0	0	0
		41	20.3	38.8	75	43	83.6	24.5	18.3	49.6
	Fungi	0	0	0	0.5	0	0.6	0.5	0.3	0.2
		1.5	0	1.3	1.5	2	1.6	2.3	2.3	1.8

* CA = *Candida albicans*; CN = *Cryptococcus neoformans*; TG = *Trichophyton gypsum*; TP = *Trichophyton rubrum* (*purpureum*).

teria, relatively few fungus colonies were present on plates at those dilutions most suitable for counting the bacteria and actinomycetes. Nevertheless, the limited data do indicate that the percentage of antagonistic forms among the fungi was much closer to that for actinomycetes than for bacteria.

The number of antagonistic actinomycetes for many of the counts

in Tables IV and V is actually greater than indicated. The reason is that often a single large zone or several confluent zones surrounded a number of actinomycetes colonies that had grown close to one another. Moreover, the actinomycetes on some plates were overgrown by soil fungi so that inhibition of the test pathogen could not be clearly discerned even if there had been antagonism. In general, nearly all of the subsurface bacterial colonies, particularly on the dextrose-asparagin agar, were very small. Nevertheless, it is unlikely for two reasons that the low percentage of active bacteria was due to limited growth. First, many actinomycetes colonies equally small in size were surrounded by fairly large zones of inhibition. Second, there were few antagonists even among the large, well-developed bacterial colonies on the surface of the agar.

From data not presented here, nearly all of twenty-two actinomycetes isolated from colonies that antagonized *Cryptococcus neoformans* in the secondary agar layer poured over soil plates, were shown to be active when streaked on the three media against the four test fungi; of fifteen actinomycetes picked from colonies showing similar inhibition against *Candida albicans*, eleven showed good activity against the pathogens when streak-tested on the three media. The others were of a low order of activity. These relatively high percentages of active microorganisms as contrasted with the corresponding values in Table I illustrate the advantage of selective procedures over random isolation in the search for antagonists. When tested by the agar streak, six of ten fungi isolated from colonies inhibitive to *Candida albicans* or *Cryptococcus neoformans* gave varying degrees of activity against the test microorganisms on the three different media. By the agar-dilution technic, only one of six cultures tested yielded filtrates that were active when heat-treated. The unheated filtrates of all six microorganisms, however, were active against *Candida albicans* or *Cryptococcus neoformans*, or both, by the cup assay. When streaked against one another, the four pathogenic fungi exhibited no inhibitive properties. It might also be mentioned that three out of six cultures of *Bacillus subtilis* selected at random and streak-tested were inactive, while three inhibited one or more of the fungi on at least one medium; three Gram-negative bacilli were completely inactive by the streak test.

SUMMARY AND CONCLUSIONS

The results of a study of various soils and compost material for the distribution of microorganisms antagonistic to fungi pathogenic for man are presented. Methods of random isolation and selective plating were employed to isolate the antagonists. The agar-streak method in which three media were employed, the agar-dilution and agar-diffusion tests with a glucose-tryptone agar were used to demonstrate antifungal activity. *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton gypseum*, and *Trichophyton rubrum* (*purpureum*) were used as the test fungi.

One hundred and twenty-four or 51 per cent of two hundred and forty-three actinomycetes were found to be antagonistic by the agar-streak method to one or more of the four test fungi. One hundred and ninety-eight of the cultures tested were isolated from various soils and forty-five were previously reported to be antagonistic to bacteriophage.

The data obtained from the selective plating procedures for isolating antifungal microorganisms show that the percentage of antagonistic bacteria was considerably less than that of the antagonistic actinomycetes. The data for soil fungi, although limited, indicate a distribution of antagonistic forms in between the values for bacteria and actinomycetes and probably closer to that of the latter.

The assays of culture filtrates of those actinomycetes active by the streak test revealed that 15 per cent of the microorganisms were active by the agar-dilution test, whereas 55 per cent exhibited inhibition by the diffusion method. The greater activity by the diffusion assay indicates this to be the more sensitive of the two methods.

The nutrient broth was decidedly inferior to the glucose-tryptone and glycerol-yeast extract media with respect to production of culture filtrates with antifungal activity, although the percentage of actinomycetes antagonistic to each of the test fungi was comparable on the three agar media used. *Cryptococcus neoformans* was found to be the more sensitive, and *Candida albicans* the more resistant to the active culture filtrates by the agar-dilution procedure.

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PRELIMINARY OBSERVATIONS ON THE MORPHOLOGY AND CYTOLOGY OF AN UNDESCRIBED HETEROBASIDI- OMYCETE FROM WASHINGTON STATE ¹

GEORGE NYLAND ²

(WITH 1 FIGURE)

A curious fungus was isolated from red raspberry leaves, severely attacked by western yellow rust (*Phragmidium rubi-idaei* (DC.) Karst.).³ In culture the fungus buds in a yeast-like manner and also produces spores by repetition. These spores, here called sporidia, are produced on sterigmata, which may develop either from similar sporidia or from the yeast-like cells. The sporidia are forcibly abjected in the same manner as the basidiospores of many Basidiomycetes.

Fifteen monosporidial cultures were made from the original isolate and all developed in the same manner. The original sporidium produced a bud within a few hours. When the budded cell attained approximately the size of the original sporidium, it separated and in turn produced a bud. Each cell so formed budded vigorously after the manner of yeasts until a colony barely visible to the unaided eye was formed. Then it was observed that, in addition to continued budding, the surface cells of the colony produced aerial sterigmata. On these, asymmetrical sporidia were formed and forcibly discharged (FIG. 1, A-D). Some of these discharged sporidia produced other cells by budding and others produced secondary sporidia on sterigmata. Apparently each sporidium is capable of doing either.

¹ Published as Scientific Paper No. 768, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman.

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³ The fungus was isolated by Dr. Folke Johnson.

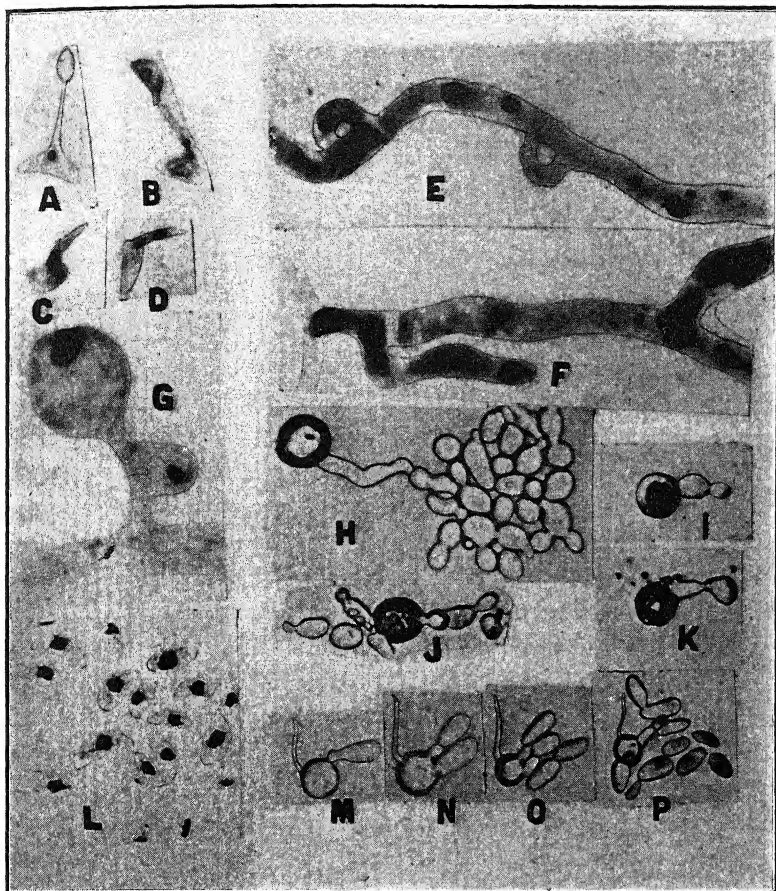


FIG. 1. *A-D*, germinating spores producing secondary spores by repetition. *D* shows 2 nuclei in the sterigma, $\times 1,400$. *E-F*, mycelium showing clamp connections and binucleate cells, $\times 1,400$. *G*, young resting spore receiving a second nucleus through the clamp connection on the stalk, $\times 1,700$. *H-K*, germinating resting spores producing yeast-like cells which later produced spores on sterigmata by repetition and also mycelium, $\times 600$. *L*, spores that were forcibly abjected from sterigmata onto a slide and stained with iron-alum haematoxylin, $\times 1,000$. *M-P*, stages in the germination of a resting spore. Photomicrographs taken at 2 hour intervals, $\times 600$.

When colonies started from a single sporidium were approximately three days old it was observed that mycelium was growing out from the margins of the colonies. The mycelium was septate, with a clamp connection at almost every septum (FIG. 1, *E-F*).

Approximately one day after the mycelium was first observed, it was noticed that resting spores were being formed some distance behind the terminal cells. These spores were produced on short stalks and were typically terminal but sometimes intercalary. A clamp connection was produced on each stalk (FIG. 1, G). Usually a hyaline, hyphal projection was observed to extend from the apex of each spore that was produced terminally. The resting spores are hyaline and thin-walled when young, but become golden brown and thick-walled when mature.

Early attempts to germinate the resting spores were unsuccessful. Germination was finally induced by incubating agar cultures at 35° C. for six days or longer. The spores germinated by producing a short germ tube or primary cell from which hyaline, yeast-like cells were budded (FIG. 1, H-K; M-P). These cells in turn budded, and development proceeded in the same manner as already described, including the formation of mycelium and resting spores.

The cells that are produced by budding, as well as the sporidia produced by repetition, are typically uninucleate (FIG. 1, L). In stained preparations occasional binucleate cells and sporidia were observed. The mycelium that develops from these colonies of predominantly uninucleate cells and sporidia is typically composed of binucleate cells (FIG. 1, E-F). The resting spores are uninucleate when young but become binucleate soon after the clamp is formed on the stalk bearing the spore (FIG. 1, G). The two nuclei in the spore fuse immediately and the spore assumes the heavy wall and golden brown color shortly thereafter. The cells formed as a result of the germination of the resting spores, and the sporidia formed subsequently, are uninucleate. It is believed that these yeast-like cells and the sporidia contain a diploid nucleus. The mycelium is believed to be dikaryotic, and fusion of the two nuclei (haploid?) occurs in the immature resting spore. Just where reduction division takes place, or if it occurs at all, is not known at the present time.

The stage in the life cycle of this fungus represented by the yeast-like cells and the sporidia agrees with the described characters of the genus *Sporobolomyces*.⁴ However, because of the presence of mycelium and resting spores, this fungus cannot be placed in this genus. Cultures of all the described species of *Sporobolomyces*

were obtained from the Centraalbureau at Delft, Holland, and subcultures have been examined repeatedly by the writer. The production of true mycelium has not been observed in any of these cultures.

Since the fungus, as far as is known, lacks the faculty of parasitism, the author hesitates to place it in the Ustilaginales. It apparently lacks an organized fruit body, at least in culture, which would seem to exclude it from the Tremellales. It has some characters in common with both orders, but, at the present and pending further study, it is simply considered to be a Heterobasidiomycete, the affinities of which are unknown. It is anticipated that in a later paper a detailed description of the fungus and a proposed name will be presented. Further study is required to provide answers to some of the puzzling features of the nuclear history.

⁴ Kluyster, A. J., and C. B. van Niel. 1924-1925. Über spiegelbilder erzeugende Hefenarten und die neue Hefengattung *Sporobolomyces*. Centralblatt für Bakteriologie, Parasitenkunde, und Infektionskrankheiten. Abt. 2, 63: 1-20.

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THE GENUS PLECTANIA AND ITS SE- REGATES IN NORTH AMERICA

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(WITH 12 FIGURES)

The genus *Plectania* was established by Fuckel (1869-70) and antedates the genus *Sarcoscypha* Saccardo by twenty years. Fuckel included two species, *Peziza coccinea* Fr. and *P. melastoma* Fr. The latter is now in the genus *Rhizopodella* Boudier. Seaver (1928) in his treatment of *Plectania* for North America included three species in addition to *P. coccinea*. Two of them, *Peziza floccosa* and *P. occidentalis*, were described by Schweinitz (1834) from specimens found in the United States, and since that date they have been collected frequently in this country. The fourth species, *Peziza protracta* Fr., is rarely collected, although it has a wide distribution to judge from published reports. It appears to be confined to the northern hemisphere, and further limited to cold areas. Only about fifty reports have been located in the literature. Six of these are from North America.

At least sixteen additional species, which have been reported from North America in the genus *Sarcoscypha*, have either already been transferred to other genera or are so inadequately described that their identity is uncertain. This investigation deals with the four species just named. It was initiated by a collection from Isle Royale, Michigan, that was made November 15, 1941, by Mr. and Mrs. H. E. Bailey. It was tentatively named *Plectania protracta* (Fr.) Gelin although it was recognized that several of its characters were distinctly different from those encountered in other species of *Plectania*. Further study, including the examination of six additional collections from both North America and Europe, together with a résumé of the literature, convinced me that this taxonomic position was untenable.

* Papers from the Herbarium of the University of Michigan.

In the course of the study, information was obtained on other species which led to the revision set forth in this paper. One phase of the study centers around the paraphyses. It was found that in the four species under consideration, they have been erroneously described or that correct descriptions have been disregarded. Paraphyses offer important diagnostic generic characters as is evident in taxonomic literature dealing with Discomycetes. In *Orbilia*, *Otidea*, *Apostemidium* and *Ionomidotis* they are universally regarded as having special significance, and in many other genera they offer much help to the taxonomist. Consequently, accurate descriptions of them are necessary.

I am greatly indebted to some of my colleagues for the opportunity of studying collections pertinent to this investigation. Thanks are due to Dr. F. J. Seaver for the loan of collections of *Plectania protracta* and *P. floccosa* from the Herbarium of the New York Botanical Garden; to Dr. J. A. Stevenson and Miss Edith Cash for slides of *Peziza floccosa* from the ex-Michener Herbarium, Bureau of Plant Industry, United States Department of Agriculture, and also for additional collections of *P. floccosa*; to Miss Ruth Patrick for fragments of *Peziza floccosa* from the Schweinitz Herbarium, Philadelphia Academy of Science. Both the slides and the fragments are presumably of the type of *P. floccosa*. The place of collection is Nazareth, Pennsylvania. Dr. Homer D. House provided the type of *Peziza Dudleyi* Peck from the Herbarium of the New York State Museum, and Dr. Rolf Singer supplied the type collection of *Sarcoscypha javensis* Syd. Miss Louise Drossall contributed specimens of *Sarcoscypha floccosa* and *S. protracta* from the Daisy Hone Herbarium in the Herbarium of the Division of Plant Industry, University of Minnesota.

KEY TO PLECTANIA AND ITS SEGREGATES

1. Paraphyses forming a reticulum.....*Anthopeziza*.
1. Paraphyses not forming a reticulum.....2.
2. Gelatinous layer present in stipe and apothecium.....*Microstoma*.
2. Gelatinous layer lacking.....*Plectania*.

PLECTANIA Fuckel

Apothecia stipitate or sometimes nearly sessile, at first subglobose, expanding to shallow cup-shaped or discoid but with margin

usually enrolled, externally somewhat hairy; hymenium bright colored inclined to scarlet; asci 8-spored; spores ellipsoid, hyaline to slightly yellowish, smooth; paraphyses forked or branched sparingly.

Type species *Peziza coccinea* Fr.

PLECTANIA COCCINEA (Fr.) Fuck. Symb. Myc. 324. 1869 (FIGS. 8-9).

Peziza coccinea Fries. Syst. Myc. 2: 79. 1822.

Lachnea coccinea Gill. Champ. Fr. Discom. 66. 1880.

Sarcoscypha coccinea Sacc. Syll. Fung. 8: 154. 1889.

Peziza Dudleyi Peck. Ann. Rept. N. Y. State Mus. 47: 23. 1894.

Geopyxis coccinea Massee. Brit. Fung. 4: 377. 1895.

Apothecia gregarious, subsessile to short stipitate, cup-shaped, margin enrolled strongly when dry, 2-4 cm. in diameter, hymenium scarlet, outside creamy white, floccose with more or less matted hyaline hairs, outside often veined and ridged when dry; stipe stout, of variable length depending upon the depth at which the sticks to which it is attached are buried; asci long cylindrical, tapering into a short stem-like base, $300-375 \times 14-16 \mu$; spores hyaline, narrowly ellipsoid, usually containing two large oil drops, $24-32(40) \times 12-14 \mu$, uniseriate; paraphyses flexuous, branched usually by forking near the base, uniformly slender, containing red coloring matter when fresh, fading on drying. No blue coloration with iodine.

On buried or partially buried sticks; early spring; North America and Europe.

MATERIAL EXAMINED: UNITED STATES. **California**: Inverness, March 1931, W. B. Cooke, (*Mich.*); Marin Co., March 20, 1939, T. T. McCabe, (*Mich.*). **Iowa**: Decorah, April 1880, E. W. Holway, *N. A. F.* 434, (*Mich.*). **Michigan**: Ann Arbor, April 1893, E. R. Wolfenden, (*Mich.*); Ann Arbor, May 14, 1893, Lola Conrad, (*Mich.*); Mason, April 1, 1929, B. Kanouse, (*Mich.*); Ann Arbor, June 1932, A. H. Smith, (*Mich.*); Lakeland, June 20, 1935, A. H. Smith, (*Mich.*); Milford, April 20, 1940, A. H. Smith, (*Mich.*); Cass Co., April 4-14, 1946, Elizabeth Halfert, (*Mich.*); Chelsea, May 19, 1947, Morten Lange, (*Mich.*). **New York**: Buttermilk Gorge, Ithaca, April 14, 1903, C. H. Kauffman, (*Mich.*). **Ohio**: Oberlin, May 1924, F. O. Grover, (*Mich.*). **Tennessee**: Tremont, March 18, 1934, L. R. Hesler, *No. 3886*, (*Mich.*); "Chimneys," Great Smoky Mts. Nat. Park, March 31, 1929, L. R. Hesler, *No. 2098*, (*Mich.*); Gatlinburg, March 27, 1940, Mrs. Jensen, (*Mich.*). **West Virginia**: Lafayette Co., March 20, 1893, L. W. Nuttall, *No. 852*, (*Mich.*).

CANADA: Old Chelsea, Quebec, April 25, 1935, I. L. Connors, (*Mich.*)

EUROPE: Bosnien, April 1901, v. Höhnelt, (Rehm: Ascomyceten, No. 1404), (Mich.).

PLECTANIA OCCIDENTALIS (Schw.) Seaver. North American Cup-fungi p. 193. 1928 (FIGS. 5-7).

Peziza occidentalis Schw. Trans. Am. Phil. Soc. II. 4: 171. 1834.

Peziza hesperidea Cooke and Peck. Grev. 1: 5. 1872.

Geopyxis occidentalis Morgan. Jour. Myc. 8: 188. 1902.

Sarcoscypha occidentalis Sacc. Syll. Fung. 8: 154. 1889.

Geopyxis hesperidea Sacc. Syll. Fung. 8: 63. 1889.

Apothecia stipitate, gregarious or cespitose, 1 cm. in diameter, shallow cup-shaped, margin enrolled when dry, hymenium scarlet, drying "capucine yellow" (R.) (1912) to "orange buff," outside smooth; stipe 1-3 cm. long, sometimes surrounded at the base with hyaline hyphae; asci cylindrical, $250-300 \times 16-18 \mu$, 8-spored, spores in one row in the asci; spores hyaline to slightly yellowish, smooth, ellipsoid, containing two large oil drops, $18-20 \times 10-12 \mu$; paraphyses flexuous, branched, forked near the base, sometimes branched more than once. No blue coloration in iodine.

On buried or partially buried sticks; type locality Muskingum, Ohio; United States.

MATERIAL EXAMINED: **Kentucky**: Harlan, Sept. 8, 1916, F. B. Cotner, (Mich.). **Maine**: Kittery Point, Aug. 1920, R. Thaxter, (Mich.), Farlow Herb., No. 641. **Michigan**: Ann Arbor, June 3, 1893, L. N. Johnson, (Mich.); Ann Arbor, May 26, 1894, L. N. Johnson, (Mich.) and Ann Arbor, Sept. 15, 1894, (Mich.); Ann Arbor, June 24, 1905, C. H. Kauffman, (Mich.); Ann Arbor, June 10, 1906, C. H. Kauffman, (Mich.); Ann Arbor, July 5, 1915, C. H. Kauffman, (Mich.); Whitmore Lake, July 25, 1915, E. B. Mains, (Mich.); Whitmore Lake, July 27, 1915, C. H. Kauffman, (Mich.); Ann Arbor, July 29, 1927, C. H. Kauffman, (Mich.); Whitmore Lake, July 10, 1929, A. H. Smith, (Mich.); Ann Arbor, July 11, 1929, B. B. Kanouse, (Mich.); Pinckney (George Reserve), July 2, 1931, E. B. Mains, (Mich.); Silver Lake, June 17, 1935, A. H. Smith, 1343, (Mich.); Sharon Hollow, June 1937, A. H. Smith, (Mich.); Ann Arbor, June 13, 1942, A. H. Smith, 18336, (Mich.). **Pennsylvania**: West Chester, N.A.F. 436. Aug. 1879, Haines, Everhart & Wood, (Mich.); Lebanon Co., June 15, 1904, C. H. Kauffman, (Mich.). **Tennessee**: Knoxville, June 25, 1929, W. A. Anderson, (Mich.). **West Virginia**: Fayette Co., Aug. 8, 1893, L. W. Nuttall, 591, (Mich.).

In the genus *Plectania* the situation regarding paraphyses as presented in the literature is confusing. A few of the contradictory citations will be briefly mentioned. To start with Fuckel's description, we find that not only the genus but also the type spe-

cies, *P. coccinea*, is credited with having filiform paraphyses. Seaver (1928) accepted this concept for the genus. Of the two species *P. coccinea* and *P. occidentalis*, respectively, he states, "paraphyses slightly enlarged above" and "paraphyses slender, slightly thickened above." His illustrations do not show branching. In an earlier paper (1904) he described and illustrated *S. coccinea* as having linear paraphyses. Of *S. occidentalis* he stated that they were linear and illustrated them as forked. In his Iowa Discomycetes (1910) the illustrations were unchanged and there were no comments concerning the paraphyses. Rehm (1895) reported *Sarcoscypha coccinea* as having paraphyses "gabelig, getheilt" and so illustrated them. Boudier (1905-1910) likewise shows them to be forked in *S. coccinea*. The American species *P. occidentalis* likewise has forked paraphyses. For the sake of accuracy this data concerning branching must be incorporated in the generic and specific descriptions, or the confusion will be perpetuated.

There remain species, still known in the genus *Sarcoscypha*, chiefly European, that should be restudied. It is possible that some described as having filiform paraphyses will be found to have them branched. A species from Java, *Sarcoscypha javensis* Syd. the type of which the writer examined, has branched paraphyses as Sydow described them.

MICROSTOMA Bernstein

Apothecia stipitate, arising from a hard, black pseudorhiza buried in the soil, usually attached to roots or wood; aerial stipe long, usually branched, covered with hairs, stipe and cups containing a layer of gelatinous hyphae; cups at first subglobose to pyriform, opening by a small pore, becoming expanded, finally laciniate; asci cylindrical, 8-spored; spores large, ellipsoid to fusoid, hyaline to slightly yellowish; paraphyses dichotomously branched several times.

Type species *Peziza protracta* Fr.

Microstoma protracta (Fr.) comb. nov.

Peziza protracta Fr. Nov. Symb. Myc. Mantissa. Act. R. Soc. Sci Uppsala 3. Ser. 1: 230. 1851. (Figs. 3-4.)

Peziza cruciata Fr. Nov. Symb. Mantissa 229. 1851.

- Microstoma hiemale* Bernst. Nova Acta Acad. Caes. Leop.-Carol. Natur. 23. 2: 649. tab. 61. 1852; Milde, J. von. Bot. Zeit. 10: 208. 1852.
- Peziza mirabilis* Borsz. Fungi Ingrici p. 61. t. 4, 5. 1857.
- Sclerotinia baccata* Fuck. Symb. Myc. 331. tab. 4. fig. 38. 1869.
- Peziza hiemalis* Karst. Myc. Fennica pars prima p. 44. 1871.
- Sclerotinia hiemalis* Fuck. Symb. Myc. Nachtr. II. p. 65. 1873.
- Scypharia coccinea* var. *hiemalis* Quél. Enchir. fung. 282. 1886.
- Lachnea mirabilis* Phill. Grev. 18: 83. 1889.
- Sarcoscypha cruciata* Sacc. Syll. Fung. 8: 154. 1889.
- Sarcoscypha protracta* Sacc. Syll. Fung. 8: 155. 1889.
- Sarcoscypha alpina* E. & E. Bull. Torrey Club 24: 281. 1897.
- Plectania hiemalis* (Nees and Bernst.) Seaver. North Am. Cup-fungi p. 193. Pl. 19. 1928.
- Plectania protracta* Gelin. Det. Kgl. Norske Viden. Selsk. Forh. Bd. 10. Nr. 52: 194. 1938.
- Plectania protracta* Imai. Bot. Mag. 52. No. 61: 362. 1938.

Apothecia stipitate, arising from an immersed, hard, elongate, pseudorhiza attached to buried wood or roots of trees, perennial, aerial stipes branched usually several times, giving rise to as many as eleven apothecia, aerial stipes long, slender, 2-6 cm. long, depending upon the depth to which the pseudorhiza is buried, lower one-half dark colored, upper portion light colored, covered with hyaline hairs, containing a layer of gelatinous tissue; cups 1-2 cm. in width, 1-2 cm. in depth, at first subglobose to pyriform, expanding, opening to deep vase-shaped, often with a flat collar, finally laciniate, opening by a small pore surrounded by a row of stiff, short, hyaline hairs, lower part of the cup covered with hyaline hairs, upper part smooth, externally bright orange-red, interior vivid rose-red, fading when dry, hypothecium consisting of a subhymenial layer of densely matted hyphae, a middle layer of gelatinous hyphae and an excipular layer the outermost tissue of which gives rise to the excipular hairs; asci cylindrical, $200-275 \times 20-23 \mu$, the base sharply contracted into long, slender hyphae originating deep within the subhymenial layer, 8-spored, operculum lateral; spores ellipsoid to fusoid, hyaline to slightly yellowish, $24-45 \times 10-14 \mu$, usually containing conspicuous globules which vary in size and number, lying obliquely in the asci, sometimes slightly overlapped; paraphyses not flexuous, dichotomously branched several times, sometimes extending beyond the asci, when fresh filled with red coloring matter (said to be stained green in iodine solution when fresh).

On buried sticks and roots. Sweden, Norway, Finland, Switzerland, Hungary, Germany, Austria, England, Scotland, Russia, Japan, Canada, Moravia, United States.

MATERIAL EXAMINED: UNITED STATES. **Colorado:** May 4, 1897, D. M. Andrew, Ellis Coll., Bethel, No. 238, (NY). **Michigan:** Isle Royale, Nov. 15, 1941, Harold and Virginia Bailey, (Mich.). **Minnesota:** Hennepin Co., May 1903, Hibbard, Daisy Hone Collection, (Minn.).

CANADA. **Manitoba:** April-May 1925, A. H. R. Buller, (Win.).

ENGLAND: Dunkeld, Perth. Potter, [Spec. George Massee Herb. (NY)].

HUNGARY: Budapest, Flora Hungarica exsiccata No. 610, (Mich.); Budapest. F. Petrak, Mycotheca generalis No. 36, (NY).

Accounts in the literature show that this species is collected in cold seasons. Schroeter (1908) and Buller (1934) state that it has been collected on frozen ground, or after the first melting snow. Usually it is found from March to early May. The Isle Royale collection, of November 15, represents a late date for fungi at that latitude.

The genus *Microstoma* was established by Bernstein for a fungus found near Breslau, Germany. He presented his paper on *Microstoma hiemale* in 1851 and publication followed (1852). Immediately following his article, in the same publication Milde (1852a) published a brief account in which he commented approvingly on Bernstein's work. Again in the same year (whether before or after Bernstein's publication no one seems to be able to decide), Milde (1852b) wrote again concerning Bernstein's collection and another one made by Nees. In this account Milde made the error of assigning Nees and Bernstein as co-authors for the species, and this slip has led to confusion regarding author citation. There is no question, however, but that Bernstein should be credited with the publication of the genus and of the species *M. hiemale*. Milde did a special service in his second report for he extended the description of the structure of the fungus to include a good discussion of the gelatinous layer. He said that it was present in the stipes and could be traced definitely up into the cups. This gelatinous character was also noted by Lloyd (1920) in connection with a collection of this fungus from the Clinton Herbarium. Lloyd wrote concerning it, "if it is gelatinous as I thought it was, I do not see how it can be classed as *Sarcoscypha*." Seaver identified this collection as *Sarcoscypha cruciata*. A slide made from this material is in the New York Botanical Garden Herbarium, but, unfortunately, it is too old and dry for one to be able to distinguish the character of the hypothecium. An attempt was made to see

the remainder of this collection which, presumably, is in the Buffalo Academy of Science Herbarium. Due to the fact that the fungi in the Clinton Herbarium have not been catalogued, the specimen (if still in existence) is not available for study. Another North American collection which I examined was *Sarcoscypha alpina* E. & E. This material was collected in Colorado by Bethel. A part of a single small apothecium is all that is left of this collection in the herbarium of the New York Botanical Garden. It is not possible to say whether or not a gelatinous layer is present. The paraphyses appear to be dichotomously branched instead of filiform as reported by Ellis. The spores are right for *M. protracta*. A collection from England in the George Massee Herbarium is in too poor condition to allow examination. The collection in the Daisy Hone Herbarium is an alcoholic preparation, is badly fragmented, and it was impossible to make a satisfactory study of it. The Canadian material from Manitoba was from the location from which Buller had taken specimens for his exhaustive study on the mechanism of spore dispersal for this species (1926, 1934). A thin gelatinous layer was found. In the Isle Royale material and in the two collections from Hungary, the gelatinous layer was well developed. It was thick and easily separable from the two adjoining layers of hymenial tissue. The gelatinous tissue was found both in the stipes and cups just as Milde (1852b) had reported it. Gelatinous tissue is not reported for species of *Plectania*, and its presence is not mentioned in descriptions of *Sarcoscypha* spp. which have not yet been transferred to *Plectania*.

Another morphological character which is unique for this species is a pseudorhiza or root-like structure from which the aerial stipes arise. It is hard, somewhat finger-shaped, and dark in color. It apparently is perennial and probably facilitates fruiting in the winter season. Bernstein called it a rhizome but the term pseudorhiza proposed by Buller (l. c.) is the better term for it. Buller stated that it "corresponds exactly to the 'rooting base' of such Hymenomycetes as *Collybia radicata*." He also stated that the second year's growth may develop a cluster of fruitbodies up to eleven in number, that this cluster may die away and a new set of fruitbodies appear which will persist until the third spring.

Heim (1925) has reported upon it as it is found in European collections.

The fungus has been found in both hardwood and coniferous woods. There is probably an attachment to tree roots. Gelin (1938) states that the "opinion is generally held that the fungus is a saprophyte, growing on pieces of decomposing branches and roots of different shrubs and trees, *Larix*, *Acer*, *Corylus*, *Populus tremula* etc."

Still a third character which calls for comment is the paraphyses. They are branched dichotomously and form compact, erect bundles. At certain stages in the development of the asci they project beyond the tips of the asci, according to Buller, and this can sometimes be observed in slides of herbarium material. Bernstein described the paraphyses as branched but, unfortunately, he did not illustrate them. They are not filiform.

The genus *Microstoma*, as described by Bernstein and extended by Milde, was established primarily upon the presence of gelatinous tissue and pseudorhiza. These, together with the type of paraphyses, warrant the transfer of *Plectania protracta* to *Microstoma*.

Peziza mirabilis, which was described by Borszczow (1857) from a collection made in Russia, is known only from his account. It is generally considered to be synonymous with *M. protracta*, but it needs further study. He did not report the presence of any gelatinous layer. His illustration of the paraphyses shows them to be rather stout, filiform and septate.

In connection with the use of the name *Microstoma*, a situation exists that needs clarification. Seaver (1928) in his synonymy of *Plectania* cited "Not *Microstoma* Bruch. 1846" which implies that the name had been used as a genus preceding Bernstein's publication. Upon investigation it was found that the name *Microstoma* as used by Bruch designated a section, not a genus, and therefore does not pre-empt the use of the name by Bernstein. Pfeiffer (1874) gives "*Microstoma* Bruch, Schimp. & Gumb. 1846. Bryol. eur. Fasc. 33-36. sect. Hymenostomi (*H. microstomum* et *squarrosus*)."

In other words it is not a synonym. The question of the validity of *Microstoma* as a generic name, as used by Bernstein, was submitted to Dr. G. R. Bisby, who gave the above solution, which agrees with that of the writer.

There is also a question of nomenclature regarding the use of the specific name *protracta* that calls for discussion. Fries (1851) published descriptions of two fungi that had been collected in Sweden. He named them *Peziza cruciata* and *P. protracta* in the order named. Gelin (1938) and Buchwald (1941) state that it is a generally accepted fact that these two fungi are identical. Buchwald states that the type of *P. protracta* no longer exists, and that *P. cruciata* is in the Herbarium at Uppsala. Gelin in establishing the combination *Plectania protracta* used the prerogative provided for in the International Rules of Botanical Nomenclature (Briquet 1935) and chose the specific name *protracta* instead of *cruciata*. This was done, apparently, on the basis of *protracta* being the name in more common usage. Gelin's decision must be accepted and the valid combination, using the genus *Microstoma*, is the one proposed here.

ANTHOPEZIZA Wettstein

Verhand. Zöol.-bot. Gesells. 35: 383. 1886.

Apothecia caespitose, subglobose becoming deep cup-shaped, externally hairy, hymenium scarlet; stipes long, slender, flexuous, hairy, sometimes branched; asci cylindrical, 8-spored; spores large, one-celled, smooth, hyaline to slightly yellowish; paraphyses repeatedly branched, anastomosing and forming a reticulum. No blue coloration with iodine.

Type: *Anthopeziza Winteri* Wetts.

The following is quoted from Wettstein (l. c.):

"*Thalamia caespitosa*, magna, longe stipitata, cum stipite flexuoso cornu speciem referentia, superne in cupulam dilatata, e mycelio denso nigrescente (non sclerotio) orta, carnosa, extus imprimis in parte inferiore lanato-pubescentia. Cupula campanulata, margine magis minusve regulariter fissio. Hymenium colore laeto. Asci longissimi, octospori. Paraphyses tenues, numerosae, apice clavatae, inter se irregulariter reticulatim connectae vel ramosae. Sporae maximae unicellulares enucleatae, 3-4 guttulatae.—Fungi terrestres, vere primo thalamia proferentes."

***Anthopeziza floccosa* (Schw.) comb. nov. (FIGS. 1-2, 10-12).**

Peziza floccosa Schw. Trans. Am. Phil. Soc. II. 4: 172. 1834.

Sarcoscypha floccosa Sacc. Syll. Fung. 8: 156. 1889.

Geopyxis floccosa Morgan. Jour. Myc. 8: 188. 1902.

Plectania floccosa Seaver. North Am. Cup-fungi, p. 192. 1928.

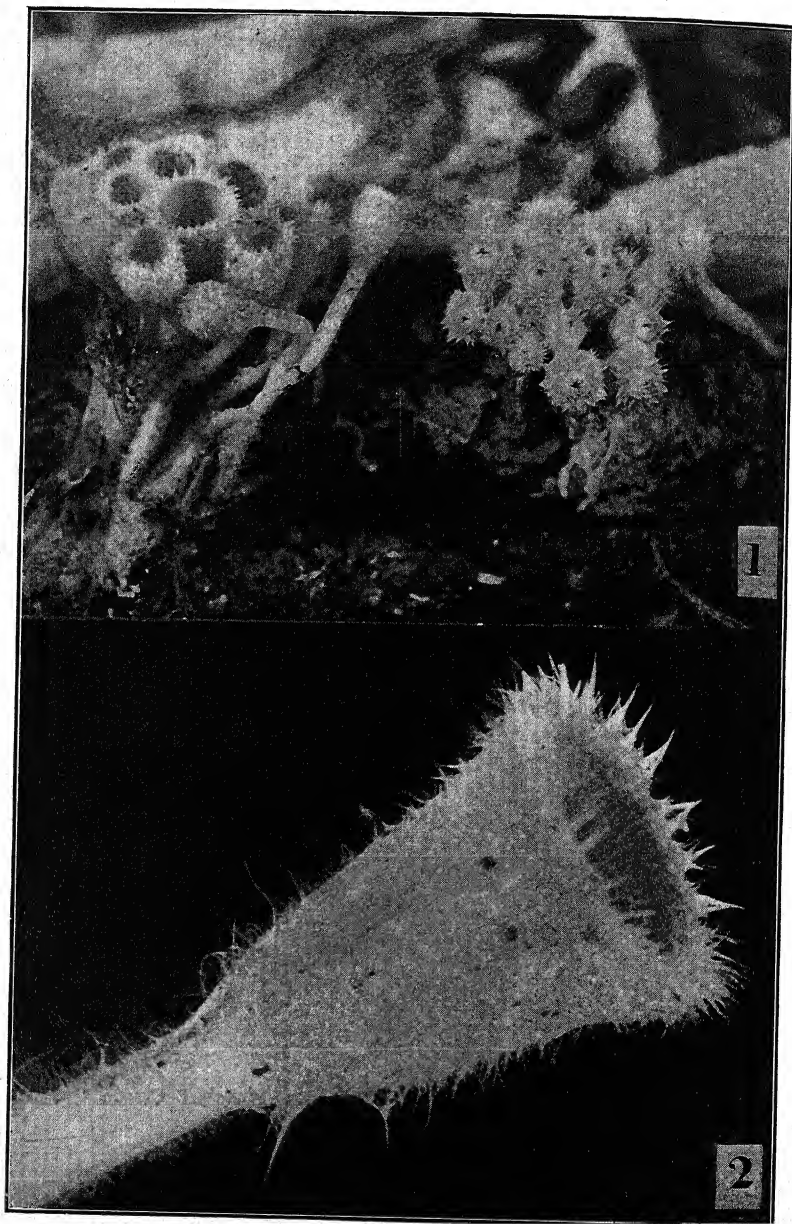
FIGS. 1-2. *Anthopeziza floccosa*.

Photo. E. B. Mains.

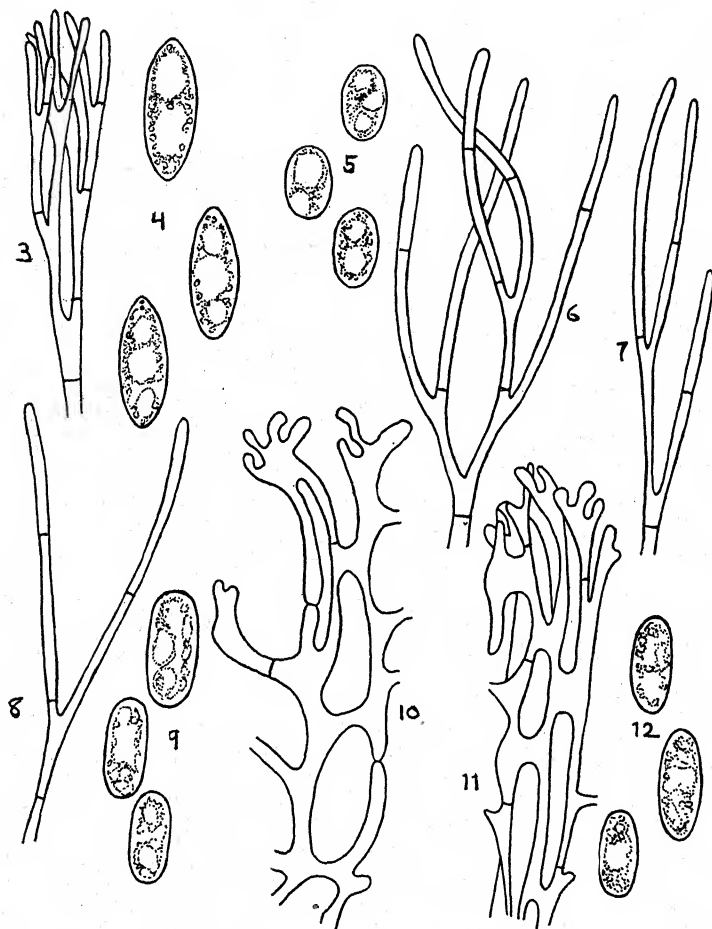
Apothecia stipitate, usually cespitose arising from buried sticks, frequently in clusters of from three to six fruitbodies, 0.5–0.75 cm. in diameter, 1–1.25 cm. in depth, subglobose, becoming inverted cone-shaped when mature, substance thin, fragile when dry, clothed with long, hyaline hairs which in the young stage form a stellate pattern over the upper portion; stipe slender, length variable up to 5 cm., covered with hyaline hairs, the lower portion dark colored, base frequently surrounded by a loose mass of hyphae; asci cylindrical, narrowed abruptly into a long stem-like base, $300\text{--}350 \times 18\text{--}20 \mu$; spores hyaline, smooth, $20\text{--}35 \times 14\text{--}16 \mu$; paraphyses branched and anastomosed forming a reticulum around the asci, small knoblike branches frequent, septate, slightly constricted at the septa, apices ending in branches that in some stages overreach the asci. No blue coloration with iodine.

Type locality: Nazareth, Pennsylvania. Distribution, United States.

MATERIAL EXAMINED: UNITED STATES. **Connecticut**: Norwich, July 4, 1890, W. A. Sturgis, (NY); Redding, July 19, 1902, Earle, No. 495, (NY). **Indiana**: Lafayette, July 13, 1930, G. B. Cummins, No. 121, (NY); Corydon, June 17, 1935, F. J. Hermann, (Mich.). **Kentucky**: Bowling Green, June 1896, I. F. Price (Herb. of Lucien M. Underwood), (NY). **Maryland**: Catoctin, June 8, 1940, J. I. Wood, (BPI). **Michigan**: Ann Arbor, July 4, 1905, C. H. Kauffman, (Mich.); Ann Arbor, July 2, 1916, E. B. Mains, (Mich.); Ann Arbor, H. M. Fitzpatrick and G. H. Smith, (NY); Ann Arbor, July 16, 1928, Gordon L. Walls, (Mich.); Ann Arbor, July 10, 1929, B. B. Kanouse, (Mich.); Pinckney, June 27, 1935, A. H. Smith, (Mich.); Chelsea, June 1937, A. H. Smith, (Mich.); Chelsea, June 27, 1937, A. H. Smith, 6411, (Mich.); Waterloo Area, June 26, 1945, A. H. Smith, 20480, (Mich.); Moscow, August 15, 1947, Bryant Walker Coll., (Mich.). **Minnesota**: Waseca Co., July 1891, Sheldon, No. 667, Daisy Hone Herb., (Minn.); Winona, June 27, 1895, J. M. Holzinger (Ellis Coll.), (NY); Groveland, July 1904, Em. Freeman (Daisy Hone Coll., No. 3103), (Minn.). **Missouri**: Perryville, 1884, C. H. Demetris (Rabenhorst-Winter, Fungi Europaei, 3171), (NY); Columbia, June 25, 1940, J. B. Routien, No. 1156, (NY). **New Jersey**: Newfield, Aug. 2, 1881, Ellis Collection, (NY). **New York**: Jamesville, June 1889, L. M. Underwood, (NY); Greenbush, Ellis Coll., (NY); Jamesville, June 1889, L. M. Underwood and O. F. Cooke, No. 79, (NY); Carrollton, Sept. 1902, C. H. Peck, (NY); Greenwood, July 6, 1930, W. S. Thomas, (NY); Long Island, Zabristne (Ellis Coll.), (NY); Herbarium of W. R. Gerard (NY). **Ohio**: Oxford, July 1, 1909, Bruce Fink, (Mich.); A Lloyd Coll. in Ellis Coll. (Ellis Coll.), (NY); Seven Caves, June 5, 1935, W. B. Cooke, No. 5036, (NY).

Pennsylvania: Nazareth, Ex-Schweinitz Herbarium (ex-Michener Herb.), (BPI), slides of type material; Nazareth, Schweinitz Herb., (PH); West Chester, Aug. 1879, Rev. M. T. Jefferis N.A.F., No. 435, (NY); West Chester, August 1879, Haines and Everhart (Ellis, No. 822), (NY);

Nazareth, June 28, 1883, ex herb. E. A. Rau, (*BPI*); Bethlehem, August 1884, E. A. Rau Rabenhorst-Winter, *Fungi Europaei*, No. 3171 Supplement, (*NY*); West Chester, June 1889, B. M. Ellis N.A.F., V (*NY*); Ohio Pyle, July 3-8, 1905, W. A. Murrill, (*NY*). **Tennessee**: Knoxville, June 1928, U. Tenn. No. 2101, (*Mich.*); La Follette, July 11, 1934, L. R. Hesler,



FIGS. 3-11. Spores and paraphyses of *Microstoma*, *Plectania* and *Anthopeziza*.

(*Mich.*); New Hopewell, May 26, 1938, A. J. Sharp, (*BPI*). **West Virginia**: Fayette Co., July 12, 1893, L. W. Nuttall, No. 1112 (*Mich.*). **Wisconsin**: Madison, 1903, R. A. & A. M. Harper (Rehm: *Ascomyceten*, No. 1776), (*NY*); Kewaunee Co., B. O. Dodge, No. 116, (*NY*); Madison, 1903, R. A. & A. M. Harper (Rehm: *Ascomyceten*, No. 1776), (*Mich.*).

Wettstein's account of the reticulate condition of the paraphyses in the genus *Anthopeziza* has been overlooked or disregarded. Some descriptions have even been altered to record the paraphyses as filiform. This has led to erroneous interpretations regarding *A. Winteri* which are to be found in several lists of synonymy. The finding of a reticulate condition in the paraphyses of *Peziza floccosa* emphasizes the importance of Wettstein's observations and lends weight to the validity of his genus. The two species *A. Winteri* and *A. floccosa* are not identical. In *A. Winteri* the paraphyses are described as having clavate tips, and they are illustrated as having a simple, regular type of reticulum. In *A. floccosa* the anastomosis is complicated and irregular, and the tips are finely branched. Wettstein's drawing shows the apothecia to be less hairy than are the cups of the North American species, *A. floccosa*. The beautifully arranged hairs over the exterior of the cups of *A. floccosa* (FIGS 1-2) are a conspicuous feature and help in spotting that fungus in the field. It is possible, of course, that *A. Winteri* is but a depauperate form of *A. floccosa*. It is desirable that further study be made on the European species. A careful search might show that the European species grows in North America. *A. floccosa*, as it was represented in the collections examined for this study, was found to be a remarkably clear-cut and distinct species with almost no variation in its morphological characters.

ANTHOPEZIZA WINTERI Wettstein, Verhand. Zöol.-bot. Gesells.

35: 383. pl. 16. figs. 1-7.

Copied from Wettstein:

Thalamia 2-10, consociationes e mycelio communi subterraneo, nigrescente, denso ortae. Thalamium initio curvato-clavatum, apice clausum; deinde longum, clavato-cornuforme, stipite duro; curvato, superne, in cupulam apertam dilatato, extus lanato-pubescenti, 3-5 cm. longum, non plicatum. Cupula initio globoso-campanulata, ore orbiculari (formam floris *Convallariae* maialis fere referens) extus glabra vel parce puberula, pallide aurantiaca, margine regulariter in dentes 8-12 subreflexos, extus parce pilosos fissio; deinde multo accrescens, circa 2 cm. longa, 1½ cm. diametro, campanulata, extus glabra, lobis marginis trigonis reflexis magnis. Hymenium intense cinnabarinum, partem anteriorem cupulae margine pallido excepto obtegens. Cupula demum non explanata, saepe irregulariter lacerata (imprimis aere humido). Asci longissimi, cylindracei, hyalini, 0.4-0.7 mm. longi, 12-16 Mikromm. diametro,

apice rotundati. Paraphyses tenues, circa 0.4–0.7 mm. longae, apice clavato-incrassatae et extus verruculis minimis obsitae, rarius indivisae, plerumque ramosae vel inter se ramulis tenuissimis connectae itaque fascies densas inter ascos formantes, in parte superiore oleo rubro intense colorato tinctae. Sporae octo, in parte superiore asci, oblique monostichae, ellipticae vel (rarius) elliptico-oblongae, hyalinae, glabrae, membrana crassa, vacuolis tribus vel rarius quatuor fide du-vel triseptatae, unicellulares, 33–35 Mikromm. longae, 11–13 Mikromm. latae.

Austria inferior. In locis umbrosis ad silvarum margines valleculae, "Oeder Saugraben" prope Rodaun; mense Martio ad nives liquescentes.

Wettstein transferred *Sclerotinia baccata* Fuck. to *Anthopeziza*. Rehm (1895) placed it in synonymy with *Sarcoscypha protracta*. Fuckel's illustration is of little help. Just where *S. baccata* belongs I am not prepared to state.

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EXPLANATION OF FIGURES

- FIGS. 1–2. Photographs of *Anthopeziza floccosa*. Photo. E. B. Mains.
FIGS. 3–4. *Microstoma protracta*; 3, a paraphysis showing dichotomous branching; 4, spores.
FIGS. 5–7. *Plectania occidentalis*; 5, spores; 6–7, branching paraphyses.
FIGS. 8–9. *Plectania coccinea*; 8, branching paraphysis; 9, spores.
FIGS. 10–12. *Anthopeziza floccosa*; 10–11, anastomosing paraphyses showing the reticulum surrounding the asci; 12, spores.

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NOTES AND BRIEF ARTICLES

HUMARIA AND LACHNEA. When several years ago an attempt was made by the writer to monograph the operculate cup-fungi of North America much difficulty was encountered in deciding upon the proper generic names to be used in many cases. Probably no two names were more difficult to unscramble than *Humaria* and *Lachnea* both of which were in general but illegitimate use at the time.

The name *Humaria* was first used by Fries (Syst. Myc. 2: 42. 1922) as a section or subgenus of *Peziza* and applied to a group of the smaller, nonvillose discomycetes. In 1889 Saccardo (Syll. Fung. 8: 118) raised Fries' subgenus to generic rank including a number of the species originally included by Fries in the subgenus. Unfortunately, however, in the meantime Fuckel (Symb. Myc. 320. 1869) obviously appropriated Fries' name but attributed it to himself, as he had done in other cases (see *Peziza* Fuckel, Symb. Myc. 306), and used it in an entirely different sense, applying it to the villose or hairy forms placed by Fries in the series or subgenus *Lachnea*. Therefore Saccardo's use of the name was invalid. The writer realizing this and in order to cause as little confusion as possible proposed *Humarina* to replace the untenable name *Humaria* of Saccardo.

The name *Lachnea* was also found to be untenable since it had previously been used for a genus of flowering plants. This was replaced by the name *Patella* of Weber in 1780, *Humaria* of Fuckel (not *Humaria* of Saccardo) being cited as a synonym of *Patella*.

Now after two decades, Dr. Bessie Kanouse of Michigan has discovered that *Patella* is untenable under the International Rules, although perfectly tenable under the American code which was in use at the time this monograph was written, and concludes that *Patella* must be replaced by *Humaria* of Fuckel. If her suggestion were to be followed, we would be placed in the embarrassing and rather ridiculous position of having the same name applied to two different genera of the discomycetes, *Humaria* (Fries) Sacc. and

Humaria Fuckel. Such a suggestion could serve no useful purpose but only add to the confusion which has already existed in the disposition of these two names. To back up her decision, eight new combinations have been proposed by her (*Mycologia* 39: 655, 656. 1947). Incidentally two of these combinations were not new at all but had been previously used and were cited as synonyms in North American Cup-fungi.

Regardless of these facts, Dr. Kanouse states "According to the International Rules of Nomenclature the name *Humaria* [of Fuckel] must be used for the species commonly relegated to the genus *Lachnea*." But must it? The same International Rules state (Sec. 2, Art. 62) "A name of a taxonomic group must be rejected if owing to its use with different meanings it becomes a permanent source of confusion or error." In our opinion this latter rule fits the case in question perfectly.

Whatever name is used to replace the untenable name *Lachnea* the use of the name *Humaria* of Fuckel cannot be justified under the International Rules. While there is nothing to prevent anyone from using any name he or she may choose it is suggested, in the light of the above facts, that the name *Humaria* be allowed to continue to quietly slumber in synonymy where it was placed, and we believe for good and valid reasons, twenty years ago.—
FRED J. SEAVER, THE NEW YORK BOTANICAL GARDEN.

SOME FUNGI COMMON TO THE HIGHLANDS OF MEXICO AND GUATEMALA AND EASTERN UNITED STATES.¹ While the writer² was doing botanical field work (June 1944 to May 1946) in Mexico and Guatemala, indiscriminate collections were made of the easily preserved fungi. About half the collections have been identified³ and of these about one third are identical with species of eastern United States.

In addition to those dried and preserved in the Herbarium of The University of Tennessee, the following species were noted in the

¹ Contributions from The Botanical Laboratory, The University of Tennessee, N. Ser.

² As a Fellow of the John Simon Guggenheim Memorial Foundation.

³ For which thanks are due Dr. L. R. Hesler, Dr. W. A. Murrill, Dr. J. A. Stevenson and Dr. J. A. Miller.

LIST OF SOME FUNGI OCCURRING BOTH IN THE HIGHLANDS OF
MEXICO AND GUATEMALA AND IN EASTERN
UNITED STATES:

PYRENOMYCETES

No. of Collections*	Fungus	Distribution as Previously Listed†
1	<i>Hypoxylon malleolus</i> Berk. & Rav.	Trop. Amer. up to Miss.
1	<i>Xylaria cubensis</i> Mont.	S.E. U.S. & Cuba

BASIDIOMYCETES

Thelephoraceae

2	<i>Aleurodiscus candidus</i> (Schw.) Burt	E. U.S. to Cal.; Mex. & Jamaica
1	<i>Stereum ochraceo-flavum</i> (Schw.)	E. U.S. to Cal.; Mex.
1	<i>Stereum rameale</i> Schw.	N.A. to Mex.; Jamaica & Puerto Rico
2	<i>Stereum sepium</i> Burt	E. U.S.; Mex.; Mass. & Wisc. to D.C. & Missouri; Colombia
1	<i>Tremellodendron merismatoides</i> (Schw.) Burt	

Polyporaceae

1	<i>Irpex farinaceus</i> Fr.	Trop. Am. & N. to Ohio and Iowa
7	<i>Polyporus abietinus</i> Fr.	N.A., Eur. & Asia
10	<i>Polyporus adustus</i> Fr.	Cosmop.
7	<i>Polyporus arcularius</i> Fr.	Conn., Fla. to Colo. & Mex.
1	<i>Polyporus australis</i> Cooke	S. U.S., trop. Amer. & trop. Asia
2	<i>Polyporus cinnamomeus</i> Fr.	Cosmop.
1	<i>Polyporus cristatus</i> Fr.	N. Central states
3	<i>Polyporus Curtisii</i> Berk.	N.Y. to Fla. & W. to Texas
1	<i>Polyporus cuticularis</i> Fr.	N. Central states
4	<i>Polyporus dichrous</i> Fr.	W. to Mo. & Kansas
1	<i>Polyporus distortus</i> Fr.	Can. & U.S. W. to Wisc. & Texas
7	<i>Polyporus gilvus</i> Fr.	Cosmop.
15	<i>Polyporus hirsutus</i> Fr.	N.A., Eur. & Asia
2	<i>Polyporus lichnoides</i> Mont.	Trop. Amer., Gulf states to Missouri
1	<i>Polyporus lucidus</i> (Leyss.) Fries	Gulf states to Conn. & Minn.
2	<i>Polyporus perennis</i> Fr.	Temp. reg. of world; in U.S. south to Va.
4	<i>Polyporus pinsitis</i> Fr.	S. Fla. & Mex. to Brazil
1	<i>Polyporus pocula</i> (Schw.) Berk. & Curt.	Ohio, Tenn., and West to Missouri
1	<i>Polyporus rhipidium</i> Berk. (<i>Favolus rhipidium</i> Berk.)	Ohio to Wisconsin
1	<i>Polyporus semipileatus</i> Pk.	E. U.S.; Maine-Fla.

* To give a crude idea of relative abundance.

† For most of the geographical information concerning the distribution of the fungi listed below, reference was made to the treatment of the Polyporaceae by Overholts, Murrill's work in the North American Flora and Burt's Treatment of the Thelephoraceae, all admittedly out of date.

BASIDIOMYCETES—Continued
 Polyporaceae—Continued

No. of Collections*	Fungus	Distribution as Previously Listed†
4	<i>Polyporus sanguineus</i> Fr.	Tropical reg. of world
1	<i>Polyporus Schweinitzii</i> Fr.	N.A., Eur. & Asia
1	<i>Polyporus sulfureus</i> Fr.	Cosmop.
7	<i>Polyporus tulipiferus</i> (Schw.) Overh.	N.A., Eur. & Asia
18	<i>Polyporus versicolor</i> Fr.	Cosmop.
8	<i>Fomes annosus</i> (Fr.) Cooke	N.A. & Eur.
4	<i>Fomes Feei</i> Fries	Fla. & trop. Amer.
9	<i>Fomes pinicola</i> Cke.	Temp. regions
1	<i>Fomes roseus</i> (Alb. & Schwein)	N. Amer. & Eur.
1	<i>Fomes senex</i> Mont.	Trop. Amer., Africa, Asia and S.E. U.S.
1	<i>Trametes malicola</i> B. & C.	Can. & N. Cen. states
5	<i>Trametes sepium</i> Berk.	Temp. N.A.
1	<i>Daedalea quercina</i> L. ex Fr.	Temp. N. Amer. & Eur.
8	<i>Lenzites betulina</i> Fr.	Temp. N. Amer. & Eur.
6	<i>Lenzites saepiaria</i> Fr. (<i>L. Berkleyi</i> Sacc.)	N. Temp. zone
<i>Agaricaceae</i>		
3	<i>Schizophyllum commune</i> Fr.	Cosmop.
1	<i>Pleurotus ostreatus</i> Fr.	Temp. N.A.
1	<i>Lactarius indigo</i> (Schw.) Fr.	E. U.S.; Vt.-Fla.
<i>Nidulariaceae</i>		
1	<i>Crucibulum vulgare</i> Tul.	U.S. & New Zealand
<i>Calostomaceae</i>		
4	<i>Calostoma cinnabarina</i> Desv.	Miss. to Penna.

field or markets: *Amanita caesaraea*, *A. chlorinosma*, *A. muscaria*, *A. rubescens*, *A. verna*; *Amanitopsis vaginata* var. *fulva* and the var. *livida*; *Cantherellus cibarius*, *C. floccosus*; *Clitocybe laccata*; *Collybia platyphylla*; *Lactarius Peckii*; *Lepiota procera*; and *Russula foetens*.

In the list presented below, some species are recorded as cosmopolitan, e.g., *Polyporus adustus*, *P. cinnamomeus*, *P. gilvus*, *P. sulphureus*, *P. versicolor* and *Schizophyllum commune*. With the possible exception of the last-named species it is my belief that they are not truly cosmopolitan. In Guatemala and Mexico they seem to be limited to the temperate regions and were not encountered on my infrequent excursions into "hot country."

Some species, e.g., *Irpex farinaceus*, *Polyporus australis*, *P. pinsitis* and *P. sanguineus*, seem to have their greatest distribution in the tropics, and probably eastern United States represents the fringe of their ranges.

Other species listed above, e.g., *Polyporus cristatus*, *P. cuticularis*, *P. semipileatus*, *Fomes pinicola*, *Trametes malicola*, *Lenzites betulina*, *Pleurotus ostreatus* and *Lactarius indigo*, have been reported seldom or not at all from south of the United States. That these and many other fungi common to eastern United States should also be growing in the temperate regions of Mexico and Guatemala is not surprising. In these Latin American highlands are many vascular species identical with, or closely related to, plants in eastern United States. Pine and oak forests, both pure and mixed with each other, abound. In addition, in these and in very heterogeneous, mixed temperate forests occur such species as: boxelder, beech, sourgum, sweetgum, redbud, basswood, sugar maple, blue beech (*Carpinus*), hophornbeam (*Ostrya*), elm, ash, holly, alder, sycamore, storax, buckthorn, dogwood, hawthorn, elderberry and wild black cherry. Other trees closely related to northern species may be found. Thus are provided living and dead substrata equal to those of eastern United States.

Moreover, between the elevations of 4,000 and 7,000 feet where most of the observations were made, the temperature extremes are those of the season in which fungi put in their most prolific appearance in eastern United States. Freezing weather is rare and on the warmest days a thermometer will seldom register over 90° F. From May to November there is usually an abundance of rain and a relatively high humidity. Thus, the substrata and the climate during half the year are such as would favor the growth of many of the fungi reported from eastern United States.

AARON J. SHARP, UNIVERSITY OF TENNESSEE.

GRAMINICOLOUS SMUTS OF NORTH AMERICA. Century I.

In the preparation of the new exsiccati series "Graminicolous Smuts of North America," I have the objective of distributing among certain institutions throughout the world authentic and representative specimens of smut fungi on grasses and cereals collected in North America. The species concept used in the naming

of these specimens is based on that used in recent published articles on graminicolous smuts by myself and co-authors. Requests have been received for illustrative specimens of several controversial species to the extent that this new exsiccati series would seem to find welcome in various herbaria throughout the world.

Most of the specimens in the first Century have been collected by myself or various loyal co-workers, and represent largely the Pacific Northwest. It is expected that future centuries will include to a greater extent other regions of the United States, as well as Canada and Mexico.

The preparation of this exsiccati series is a cooperative project, involving the Washington Agricultural Experiment Station, Pullman, Washington; and the Divisions of Mycology and Disease Survey and Forage Crops and Diseases, of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture. With a view toward wide geographic distribution, complete sets are being deposited in the following herbaria: Mycological Collections, Bureau of Plant Industry, Beltsville, Md.; Department of Plant Pathology, Washington State College, Pullman, Washington; Farlow Cryptogamic Herbarium, Cambridge, Mass.; New York Botanical Garden, New York; University of Michigan, Ann Arbor, Mich.; University of California, Berkeley, Calif.; Division of Botany and Plant Pathology, Central Exper. Farm, Ottawa, Canada; Spegazzini Botanical Institute, La Plata, Argentina; Commonwealth Mycological Institute, Kew, England; Institute of Systematic Botany, University of Uppsala, Uppsala, Sweden; Museum of Natural History, Paris, France; Institut de Botanique et Herbier Boissier, Geneva, Switzerland; Herb. Crypt. Ind. Orientalis, New Delhi, India; Department of Agriculture, Sydney, New South Wales.

I should be very grateful to receive collections of graminicolous smuts, especially on grasses, from any part of North America in sufficient quantity for inclusion in this exsiccati series. Full credit will be given to the collectors in every instance.—GEORGE W. FISCHER.

LABORATORY DIAGNOSIS OF MYCOTIC DISEASES

1. A refresher course for laboratory personnel in the Laboratory Diagnosis of Mycotic Diseases will be offered at the Laboratory Division of the Communicable Disease Center. The first course will be given from August 30 to September 24, 1948.

2. This training is open to all grades of employed laboratory personnel. Although first consideration will be given to the laboratories of state and local public health departments, applicants from hospitals and private laboratories will be considered when vacancies occur.

3. There is no tuition or laboratory fee but travel and living expenses must be paid for by the individual or his employer.

4. Applications for the course should be made as far in advance as possible. Notification of acceptance from this office will be made in sufficient time to allow the students to make arrangements for living accommodations. It is suggested that trainees obtain reservations for living accommodations at the earliest possible date. A list of hotels and rooming houses will be sent to applicants at the time of acceptance.

TENTATIVE OUTLINE FOR THE FOUR-WEEKS COURSE IN THE
LABORATORY DIAGNOSIS OF MYCOTIC DISEASES

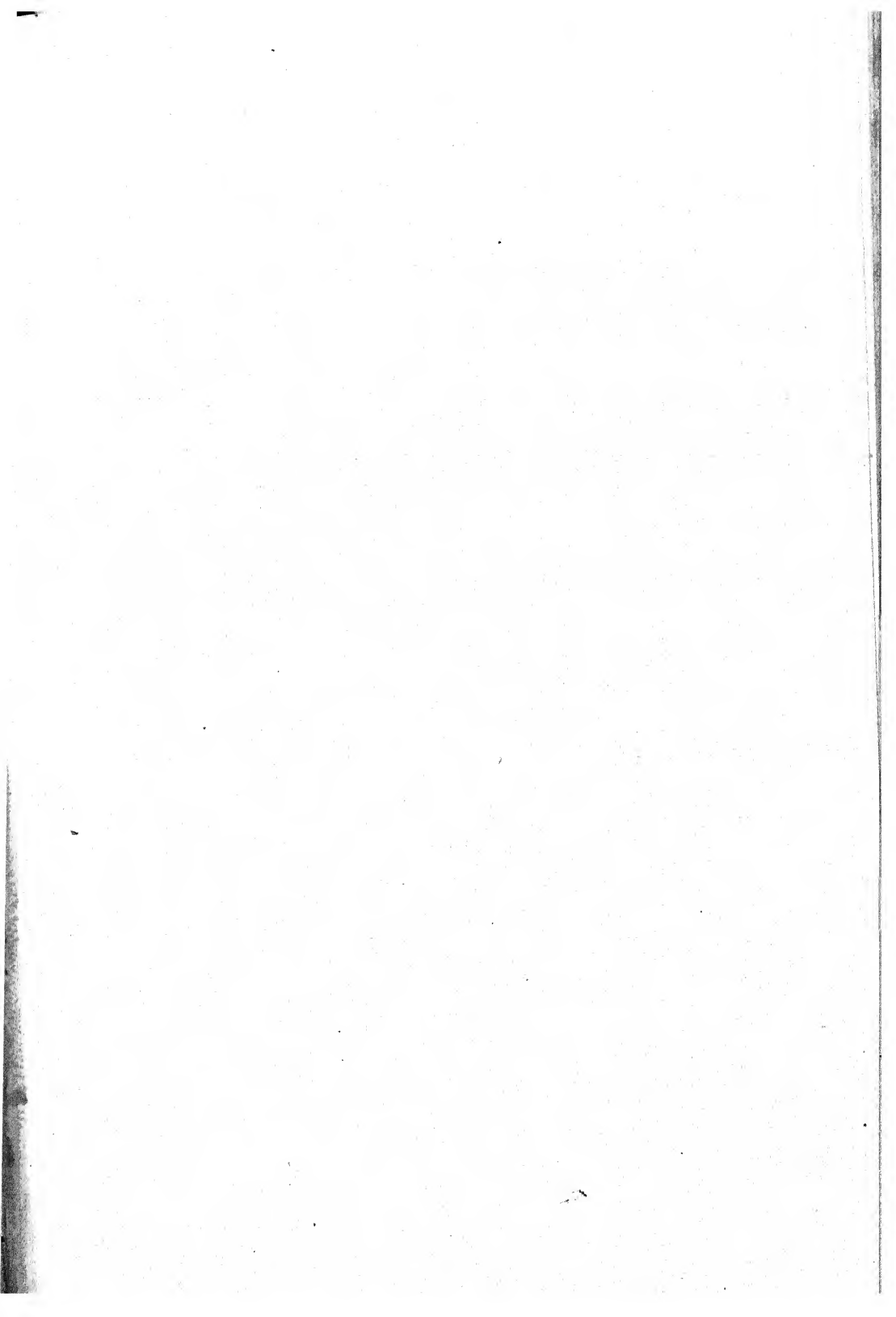
Phases of the program and relative amount of time allotted to each is as follows:

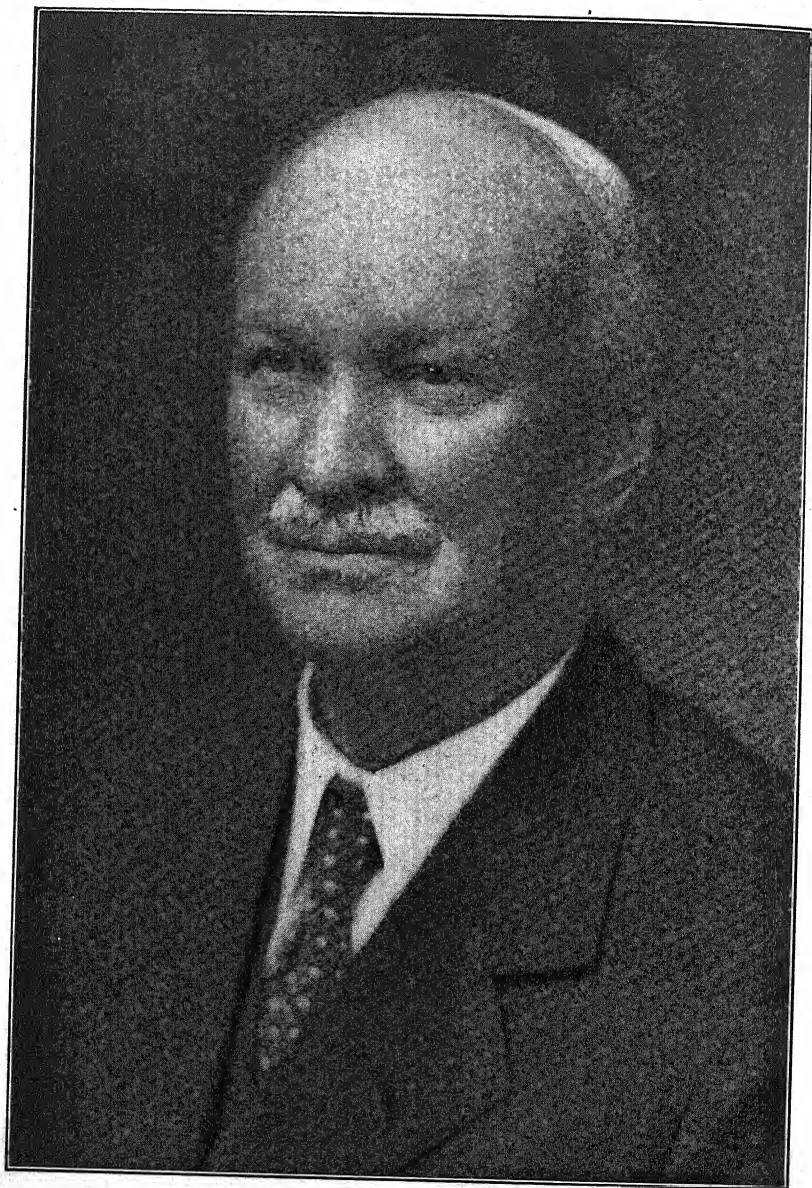
- | | |
|--|--------|
| 1. Identification of common saprophytes | 3 days |
| <i>Aspergillus, Penicillium, Cephalosporium, Fusarium, etc.</i> | |
| 2. Identification and culturing of the dermatophytes | 5 days |
| <i>Trichophyton, Microsporum, Epidermophyton, etc.</i> | |
| 3. Identification and culturing of the sub-cutaneous fungi | 6 days |
| <i>Hormodendron, Phialophora, Sporotrichum, Allescheria, Nocardia, Actinomyces, etc.</i> | |
| 4. Identification and culturing of the systemic fungi | 6 days |
| <i>Coccidioides, Histoplasma, Blastomyces, Cryptococcus, etc.</i> | |

Stress will be placed on practical laboratory procedures useful for establishing a diagnosis of mycotic infection, including the following:

1. Isolation techniques.
2. Preparation and use of special culture media.
3. Fermentation reaction tests.
4. Vaccine preparation.
5. Agglutination and complement fixation tests.
6. Inoculation of animals.
7. Preparation of permanent mounts.
8. Slide cultures.

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HENRY CURTIS BEARDSLEE

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XL SEPTEMBER-OCTOBER, 1948 No. 5

HENRY CURTIS BEARDSLEE

GERTRUDE S. BURLINGHAM

Mr. Henry Curtis Beardslee, one of the leading mycologists in the United States, died at the age of eighty-two on January 1st, 1948, in Painesville, Ohio, the place of his birth. His interest in Nature and Botany began with the teaching of his father, a physician and botanist, who went with a group of Connecticut colonists to settle in the Western Reserve in Ohio. The Beardslees were of English and Scottish descent.

After graduation from the Painesville High School in 1883, Mr. Beardslee attended Oberlin College for one year before entering Adelbert College of Western Reserve University in Cleveland, Ohio, from which he was graduated with the degree of A.B. In 1892 he received the A.M. degree. Upon graduation he entered the teaching profession, teaching one year in the Academy at Green Springs, Ohio, following which he became teacher of Science in the University School in Cleveland, Ohio, where he remained eleven years.

In 1893 Mr. Beardslee was married to Miss Anna A. Ford, a graduate of Oberlin College. Mrs. Beardslee was much interested in his mycological work and in his teaching. After her death in November 1946, he wrote "I had fifty years of wonderful companionship and could not have been happier."

In 1901 with two associates he established the Asheville School for Boys where he remained as Senior Master until his retirement in 1919. During this time he gave intensive study to the fungous flora of the vicinity and in 1918 he published in the Journal of the Elisha Mitchell Scientific Society a Monograph on "The Rus-

[MYCOLOGIA for July-August (40: 391-504) was issued July 29, 1948]

sulas of North Carolina" illustrated by forty-one photographs of natural size, twenty-three of which were taken by himself, and the remainder by Prof. W. C. Coker of the University of North Carolina. Among the species were three described for the first time, *Russula cinerascens*, *R. pungens* and *R. magna*, also *R. rubescens* which had been previously described by him in *Mycologia* 6: 91. '14. On the plate of spore drawings of twenty-eight species he brought out the patterns of reticulations which had not been shown in spore drawings. These drawings were made without the Crawshay iodine method which was published by Crawshay in "Spore Ornamentation of the Russulas" in 1930.

Mr. Beardslee was a very careful scientist, always obtaining the taste of the species of *Russula* in the field, and looking for any color changes in the flesh, and for any odor either in the fresh state or as it might develop later. The same care was given to collections of species in other genera. He spent one summer in Germany and at another time traveled over Europe during which time he met leading mycologists. In Sweden he had the privilege of collecting with Lars Romell, and with him going over the collecting grounds so familiar to Elias Fries.

After his retirement in 1919 he spent the winters in Florida, first on the East Coast, then in Central Florida at Longwood and Altamonte Springs. During this time he made extensive collections in many genera of fleshy fungi, accompanying the species with full notes, photographs and spore prints. Before his death he presented his herbarium to Oberlin College. Each year after the season for fleshy fungi was over he began to enlarge his herbarium of flowering plants.

In *Mycologia* 32: 575-586. 1940 he cooperated with the writer in publication of seven new species of *Lactaria*.

Mr. Beardslee was a member of the American Association for the Advancement of Science, the Botanical Society of America, the Torrey Botanical Club, The Mycological Society of America, The British Mycological Society, and Phi Beta Kappa.

Not only did Mr. Beardslee make a lasting contribution to Mycology, but his influence as a scholar and a Christian gentleman will be passed on through the character of the men whom as boys he taught in the Asheville School in Asheville, North Carolina.

NEW SPECIES OF *PENICILLIUM*

KENNETH B. RAPER¹ AND DOROTHY I. FENNELL²

(WITH 11 FIGURES)

Through the cooperative effort of the United States Department of Agriculture and the National Science Fund, a comprehensive study of the genus *Penicillium* has been conducted at the Northern Regional Research Laboratory, during the past three years, leading to the preparation of a manual covering this important genus of molds (Raper and Thom, in press). An enormous number of *Penicillia* were examined culturally and microscopically, and in the course of this work eleven species and one variety which are believed to be new have been encountered. Both ascosporic and non-ascosporic forms are represented.

Cultures of species types are being deposited with the American Type Culture Collection, Washington, D. C.; the Centraalbureau voor Schimmelcultures, Baarn, Holland; and the collection maintained at The London School of Hygiene and Tropical Medicine.

All of the species currently described have been under observation for a period of at least one year, and some for more than five years. They have been grown upon a wide variety of culture media including Czapek's solution agar and various modifications of the same, malt extract, Sabouraud's dextrose, corn meal, and hay infusion agars. All of the forms grow well at room temperatures of 23 to 25° C. Since the development of diagnostic characteristics in certain of these new species seems to be dependent upon the use of particular substrata, the compositions of the media employed in this study are given.

¹ Principal Microbiologist, Fermentation Division, Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

² Assistant Microbiologist, National Science Fund, and Cooperative Agent, Northern Regional Research Laboratory.

(1) *Czapek's solution agar*: Distilled water, 1 liter; sucrose, 30 gms.; NaNO_3 , 3.0 gms.; K_2HPO_4 , 1.0 gm.; KCl , 0.5 gm.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 gm.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 gm.; agar, 15 gms. The reaction is near neutral and is not adjusted.

(2) *Steep agar*: Czapek's solution agar plus the addition of 10 ml. concentrated corn steep liquor (ca. 50 per cent solids). The reaction is adjusted to pH 7.0 with NaOH before sterilization.

(3) *Malt extract agar*: Distilled water, 1 liter; malt extract (Difco), 20 gms.; dextrose, 20 gms.; peptone, 1.0 gm.; agar, 25 gms. The pH is approximately 4.7 and is not adjusted.

(4) *Corn meal agar*: Boil 50 gms. white corn meal (contained in cloth bag) in one liter distilled water for one-half hour, filter, and make up to original volume; add 15 gms. agar and sterilize. The reaction is approximately pH 8.0 and is not adjusted.

(5) *Hay infusion agar*: Cook 50 gms. decomposing hay in one liter of distilled water in autoclave for 30 minutes, filter. Add 2 gms. K_2HPO_4 and 15 gms. agar per liter of infusion filtrate, adjust reaction to pH 6.2 with HCl and sterilize.

(6) *20 per cent sucrose Czapek*: Similar to (1) above except containing 20 per cent rather than 3 per cent sucrose. The reaction is near neutral and is not adjusted.

(7) *Ammonium sulfate Czapek*: Similar to (1) above except containing 2.33 gms. $(\text{NH}_4)_2\text{SO}_4$ rather than 3.0 gms. NaNO_3 . The reaction is approximately pH 6.8 and is not adjusted.

(8) *Sabouraud's dextrose agar*: Distilled water, 1 liter; peptone, 10 gms.; dextrose, 40 gms.; agar, 20 gms. The reaction is pH 5.6 to 5.7 and is not adjusted.

ASCOSPORIC SPECIES

Penicillium parvum sp. nov.

Coloniae in agaris omnibus valde restrictae; e coacta densa compositae vel pertenuae, mycelio vegeto plurimum submerso, primum albae, demum sordide flavae usque pallide brunneae vel vinaceae; reverso vinaceo usque brunneo-rubro, fructificationibus conidicis sparsis; conidiophoris brevibus, vulgo $25\text{--}40 \times 1.5 \mu$, glabris; penicillis parvis, constanter monoverticillatis; sterigmatibus in 3-6 verticillis parallelibus, $7.0\text{--}8.0 \times 1.2\text{--}1.5 \mu$; conidiis primum ellipticis, $1.5\text{--}2.0 \times 1.2\text{--}1.5 \mu$, in aetate subglobosis, breve catenatis; peritheciis sphaericibus vel oblongis, plerumque 100μ in diam. non excedentibus, non-ostiolatis, alutaceis usque pallide brunneis, primum sclerotioideis, a centro extus tarde maturescentibus, peridio integro cellulas duas vel tres crasso; ascis globosis vel ovalibus, $6.0\text{--}7.0 \mu$ in diam., octosporis; ascosporis lenticularibus, parvissimis, $2.0\text{--}2.4 \times 1.5\text{--}1.8 \mu$, distincte asperatis et costis prominentibus equatorialibus praeditis.

In culturis e solo, Nicaragua.

Colonies on Czapek's solution agar growing very restrictedly, attaining a diameter of 1.5 to 2.0 cm. in two weeks and 3 to 4 cm. in four to five weeks, raised 1 to 2 mm., sometimes folded or wrinkled

(FIG. 1A), consisting of a very tough close-textured mycelial felt with surface characterized by a thin growth of fine aerial hyphae appearing somewhat floccose, mostly in white through flesh to dull yellow shades, becoming deeper and somewhat vinaceous (Ridgeway, Pls. XXVII and XL) in age, penicilli usually lacking (see hay agar); incipient perithecia developing within two to three weeks, spherical to oblong or slightly angular, yellow in color, buried deep

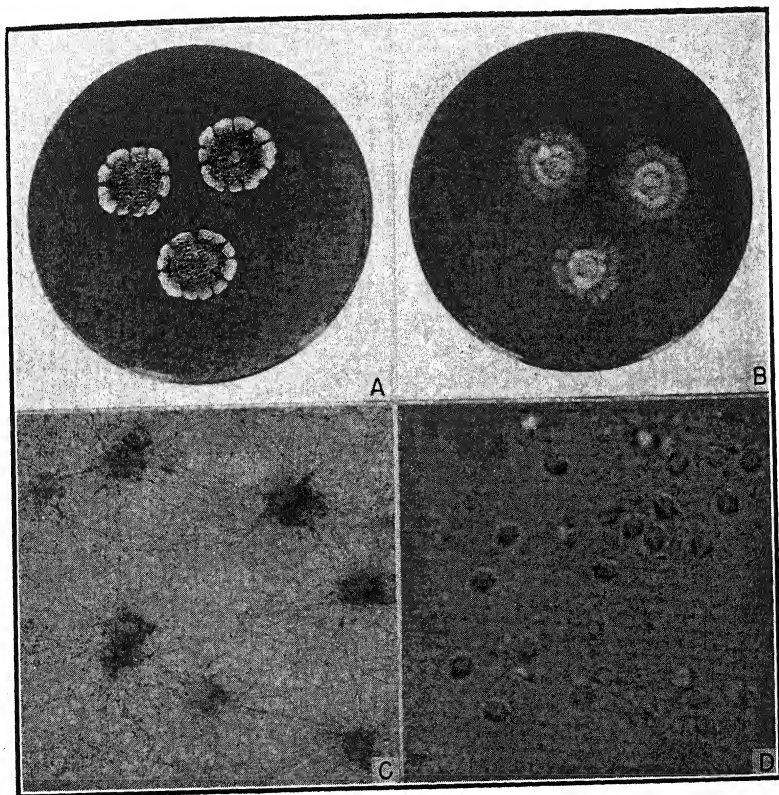


FIG. 1. *Penicillium parvum*.

in the mycelial felt, failing to produce asci and ascospores even within four to five weeks; exudate usually abundant, in rich brown shades becoming deep purple-brown in age; odor lacking or indistinct; reverse at first in vinaceous fawn shades becoming deep maroon in age.

Colonies on steep agar restricted but growing more rapidly than above, 2.0 to 2.5 cm. in two weeks, in texture and appearance essentially as on Czapek but with surface of looser texture and

deeper; exudate more abundant, deep maroon; penicilli usually lacking; not developing asci and ascospores within one month.

Colonies on malt agar about 1.5 to 2.0 cm. in two weeks, 0.5 to 1.0 mm. deep, plane or nearly so, consisting of a dense layer of perithecia adjacent to the agar surface, commonly overgrown by a thin, loose network of orange to light brown aerial hyphae, lending to the colony its characteristic texture and color; penicilli very few in number, strictly monoverticillate, on short branches from submerged or trailing hyphae, not affecting the colony appearance; no exudate or odor; reverse in dull brown shades.

Colonies on hay infusion agar restricted, about 2.5 to 3.0 cm. in three to four weeks, very thin, vegetative mycelium largely submerged, producing numerous perithecia in an uneven layer at the agar surface to give a granular effect (FIG. 1B), in tan to brown shades; penicilli very few in number, developing primarily at the colony margins in old plates, strictly monoverticillate, borne on short branches from submerged or trailing hyphae; conidiophores short, usually $50\ \mu$ or less and commonly 25 to $40\ \mu$ in length by about $1.5\ \mu$ in diameter, smooth-walled; penicilli consisting of verticils of 3 to 6 parallel sterigmata; sterigmata mostly 7 or $8\ \mu$ by 1.2 to $1.5\ \mu$ with conidium-bearing tips slightly narrowed; conidia at first definitely elliptical, about 1.5 to $2.0\ \mu$ by 1.2 to $1.5\ \mu$, in age becoming almost subglobose, mostly 1.5 to $1.8\ \mu$ in diameter, smooth-walled, adhering in fairly long chains in fluid mounts. Perithecia spherical to oblong (FIG. 1C), mostly $100\ \mu$ or less in diameter, occasionally up to $150\ \mu$, surrounded by very thin wefts of sterile hyphae, at first tending to be sclerotoid and of uniform texture throughout, consisting of heavy-walled pseudoparenchyma, ripening late and from the center outward, beginning to develop asci and ascospores in three to four weeks, at two months filling the perithecium except for an outer wall 2 to 3 cells thick; asci apparently borne as lateral branches from fertile hyphae, chains not seen, round to oval in outline, about 6 to $7\ \mu$ at maturity, 8-spored; ascospores lenticular, very small, 2.0 to $2.4\ \mu$ by 1.5 to $1.8\ \mu$, with walls definitely roughened and with two prominent equatorial ridges rather widely separated to give a definite pulley-like appearance (FIG. 1D).

Colonies on corn meal agar spreading slowly, 5 to 6 cm. in four weeks, very thin, vegetative mycelium submerged; perithecia produced abundantly along radiating dendroid lines, surrounded by very sparse hyphal networks, developing and ripening as on hay agar; penicilli usually absent.

Species description based upon NRRL 2095 as type, isolated in July 1945 from a sample of soil from Nicaragua contributed by Dr. A. G. Kevorkian.

The binomial, *Penicillium parvum*, was selected because of the minute character of the penicilli, conidia, and ascospores. *Penicillium minutum* would have constituted a more suitable name, but the describers refrained from adopting this because of Bainier and Sartory's (1913) use of the name *Citromyces minutus* for an apparently strictly conidial form.

Penicillium parvum is believed to be more nearly related to *P. javanicum* van Beyma (1929) than to any other described species. It resembles the latter in producing colonies marked by a rich reddish brown pigmentation in reverse; in showing strictly monoverticillate penicilli borne on short branches; and in developing perithecia—at first sclerotoid in character—which subsequently ripen from the center outward. It differs from this species in its more restricted growth upon all media, particularly upon Czapek's solution agar; in the smaller dimensions of conidial structures and parts of the same; in the more delayed ripening of its perithecia and, particularly, in the character of its ascospores. These are consistently smaller, more conspicuously roughened and show more strongly developed equatorial ridges and furrows. The species is represented, at present, by a single strain.

Penicillium parvum is apparently favored by a culture substrate of high osmotic tension. It grows better upon all substrata as these dry out in the culture plate or tube, producing conidial structures on most media only in marginal areas of aging cultures. The production of penicilli upon agar media to which a high concentration of NaCl has been added affords additional evidence. Growth of the fungus upon Czapek's solution agar is increased when the sugar concentration is raised to 20 per cent.

Penicillium levitum sp. nov.

Coloniae in agaro Czapekii satis restrictae et coactae dense texta, valde rugosa efficientes, in agaro maltoso effusae, tenues planaeque, primum albae, plerumque vetustae bubalinae vel alutaceae; reverso flavo; fructificatione conidica sparsa; conidiophoris plerumque ex hyphis aereis, 20–50 × 2.0–2.8 μ , glabris; penicillis monoverticillatis, parvis; sterigmatibus saepe in 3–5 verticillis simplicibus, interdum geminatis vel singulis, plerumque 7.0–12.0 × 2.2–3.3 μ ; conidiis globosis vel ovalibus, 3.0–8.0 μ in diam., plerumque 4.0–6.0 μ , glabris, brevemente catenatis; peritheciis in agaro maltoso et milii indici abundantibus, globosis vel subglobosis, usque 100 μ diam., pallide alutaceis, primum pseudoparenchymatibus, ascis et ascosporis aream centralem implentibus,

peridio cellulari, cellulas 1-2 crasso; ascis singulis, ovalibus vel oblongis, 8-10 μ in diam., octosporis; ascosporis late ellipticis vel subglobosis, 3.5-4.5 \times 3.0-4.0 μ , glabris, costis rimisque aequatorialibus carentibus.

In culturis ex argilla, New York.

Colonies on Czapek's solution agar growing rather restrictedly, about 3.0 to 4.0 cm. in two to three weeks at room temperature, comparatively thin, consisting of a close felt tearing with difficulty, central area raised, often pulling away from the culture dish and usually splitting along deep radial furrows (FIG. 2A), surface appearing almost velvety but consisting of a thin network of short, closely interwoven vegetative hyphae, white but gradually developing light buff to flesh shades in marginal areas, in age sometimes showing yellow and lilac zones, conidia limited, borne on very reduced fruiting structures (see 20 per cent sucrose Czapek agar) commonly consisting of single sterigmatic cells, not influencing the colony appearance; perithecial primordia present in limited numbers (see malt agar), buried in the mycelial felt, not developing mature asci or ascospores and not affecting the colony appearance; exudate limited, clear; odor lacking; reverse in yellow shades from citrine to dull buff.

Colonies on Czapek's solution agar containing 20 per cent sucrose as described above but developing conidial structures and perithecial initials more abundantly; conidiophores arising as short branches from aerial hyphae, smooth-walled, mostly 20 to 35 μ by 2.0 to 2.8 μ , often shorter and rarely longer than 50 or 60 μ ; penicilli consistently small, simple (FIG. 2C); sterigmata rarely more than 4 or 5 in the verticil, commonly irregularly arranged, often occurring in pairs or singly, variable in size, mostly 7 to 12 μ by 2.2 to 3.3 μ when borne in clusters, up to 20 to 25 μ by 3.0 to 3.5 μ when arising singly, showing some tendency to be wedge-shaped but narrowing to conidium bearing tubes; conidia globose to subglobose, oval or somewhat pyriform with comparatively heavy, smooth walls, varying greatly in dimensions (FIG. 2C), mostly 4.0 to 6.0 μ in diameter but ranging from 3.0 to 8.0 μ , borne in very short divergent chains, consistently larger in some chains than in others.

Colonies on steep agar growing as on Czapek but less deeply furrowed, producing abundant perithecia (largely near the colony surface) which ripen within 10 days to 2 weeks.

Colonies on malt agar spreading broadly, attaining a diameter of 6 cm. in two to three weeks, plane, occasionally zonate, growing margin 0.5 to 1.0 cm. wide, white to light flesh colored, central area in shades near avellaneous from the abundant perithecia borne in a loose mycelial felt; conidial structures lacking or very limited in

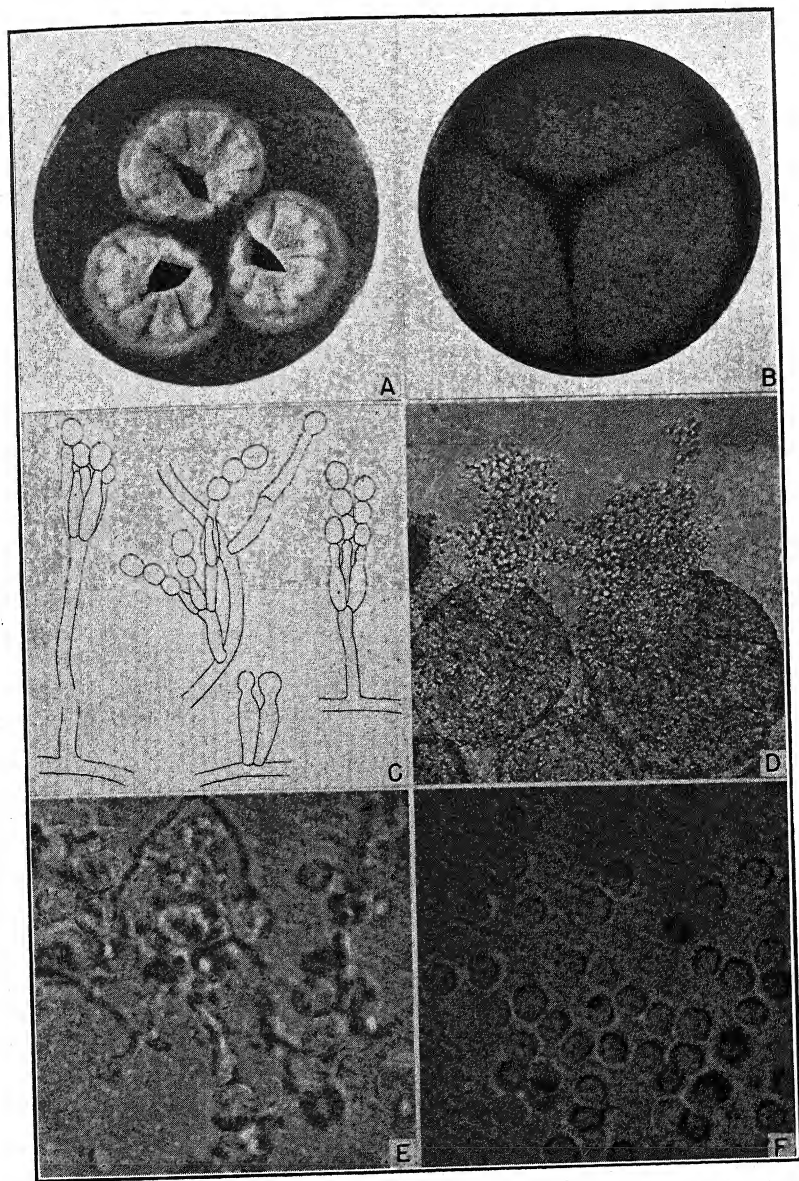


FIG. 2. *Penicillium levitum*.

number; perithecia spherical or nearly so, mostly 50 to 100 μ in diameter, in light tan shades, at first consisting of pseudoparenchyma throughout but quickly developing fertile tissue in central areas; asci evident within 4 to 5 days and ascospores beginning to ripen within a week, fertile area progressing outward and within 10 days to 2 weeks filling the entire perithecium except for a thin peridium (FIG. 2D) one to two cells thick; asci borne as short branches from fertile hyphae (FIG. 2E), not in chains, spherical to oval or oblong, 8 to 10 μ in diameter when mature, 8-spored; ascospores smooth, polished, broadly elliptical to subglobose, 3.5 to 4.5 μ by 3.0 to 4.0 μ , comparatively heavy-walled, without any indication of equatorial furrow or ridges (FIG. 2F).

Colonies on corn meal agar spreading broadly, up to 6 cm. in two to three weeks (FIG. 2B), very thin, vegetative mycelium submerged or forming a loose network at the agar surface; perithecia rather sparsely produced, with form, dimensions, and development as on malt agar; conidial stage lacking or very limited.

Species description based upon NRRL 705 as **type**, received without name from Dr. B. O. Dodge in 1936 as an isolate from modeling clay. This culture has been maintained in our collection since that date as an unidentified member of the general series with *Penicillium brefeldianum* Dodge (1933). Careful examination of this culture during our current study and detailed comparison with described species lead us to regard the form as new. The binomial, *P. levitum*, from the Latin *lēvitas* (smoothness), is applied because of the conspicuously smooth character of all walls, especially those of conidia and ascospores.

Upon most substrata, and particularly those containing vegetable extracts, the stock strain of *Penicillium levitum* produces abundant perithecia but very few sterigmatic cells, either grouped as simple penicilli or arising singly from aerial hyphae. Sector variants characterized by increased conidium formation and an absence of perithecia are occasionally observed, and sub-cultures derived from these seem to maintain the characteristics of the sectors. Conidia are produced fairly abundantly and upon some substrata, *e.g.*, 20 per cent sucrose Czapek, the colony surface may assume a light, pale blue-gray tint. While the number of conidia produced in such variant sub-strains is much greater than in the stock, the penicilli produced are not more complex and seldom show clusters of more than

4 or 5 sterigmata. Measurements of conidia and sterigmatic cells remain unchanged.

The perithecia of *Penicillium levitum* are less highly specialized than the initially sclerotoid structures which characterize such monoverticillate species as *P. javanicum* v. Beyma and *P. ehrlichii* Klebahn (1930). Conidial structures also differ from other members of the series. Whereas 4 or 5 sterigmata may be arranged in a simple verticil, meeting the essential requirement for placement in the genus *Penicillium*, such definite structures are not consistently produced and the total conidial picture is strongly suggestive of the genus *Monascus*. Such relationship is further suggested by the fact that ascus walls break down rather quickly, leaving the spores free within the ripening perithecium. The species is regarded as properly assignable in the genus *Penicillium*, but somewhat transitional in the direction of *Monascus*.

Perithecia of *Penicillium levitum* ripen more rapidly but apparently follow the same basic pattern of development shown by *P. javanicum* v. Beyma. The young perithecium rapidly assumes its ultimate size and appears pseudoparenchymatous throughout. A mass of fertile tissue soon develops in the central area and, in contrast to two or more weeks in such species as *P. javanicum* and *P. parvum*, asci may appear as early as the 4th or 5th day. The mass of asci and ascospores usually fills the perithecium within two weeks or less and a thin peridium one or two cells thick confines the ascospores at maturity. The perithecium is hardly firm at any stage and is certainly not sclerotoid, but the continuity of the pseudoparenchymatous tissue when young and the presence of a definite continuous wall at maturity seem to relate it unquestionably to *P. brefeldianum* Dodge and *P. javanicum* v. Beyma.

Penicillium helicum sp. nov.

Coloniae in agaro Czapekii multo restrictae, comparative tenues, laxae textae, fructificationes conidicas paucas et perithecia rara gerentes; in agaro maltoso late crescentes, peritheciis abundantibus, aureo-flavae, reverso auran-tio-rubro; conidiophoris $75-100 \times 2.0-2.5 \mu$, glabris vel irregulariter asperatis; penicillis typice biverticillatis et symmetricis, sed saepe non ab omni parte vel monoverticillatis; metulis in 3-4 verticillis $10-15 \times 2 \mu$; sterigmatibus lanceolatis, apicibus acuminatis, in 6-7 verticillis $8.0-12.0 \times 2.0-2.5 \mu$; conidiis ellipticis, $3.0-3.5 \times 2.5 \mu$, glabris; peritheciis laxae textis, mollibus, ex hyphis intertextis intense coloratis limitatis, circa $200-250 \mu$ in diam., saepe conflu-

entibus; ascis catenatis, sphaericalibus usque ovatis, $5.5-7.0\ \mu$, octosporis: ascosporis ellipticis superficiem totam spinulosis, $2.5-3.0 \times 1.4-1.8\ \mu$.

In culturis e solo, Suecia.

Colonies on Czapek's solution agar growing restrictedly, commonly not exceeding 1.5 to 2.0 cm. in two to three weeks at room temperature, comparatively thin, with vegetative mycelium largely submerged and with surface growth comparatively loose, almost

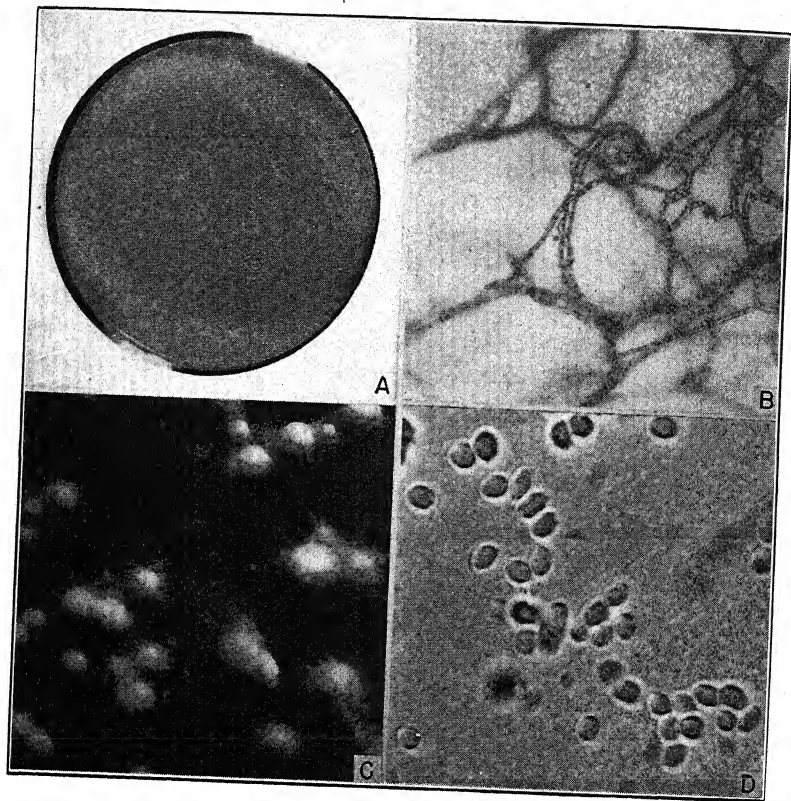


FIG. 3. *Penicillium helicum*.

floccose, in flesh to orange-pink shades, tardily developing conidial structures in limited numbers; rarely producing perithecia (see malt agar); odor not pronounced; exudate limited or lacking; reverse at first colorless, developing red shades in age; penicilli variable in pattern, mostly fractional, commonly appearing monoverticillate, sometimes branched and asymmetric, sometimes biverticillately symmetrical in the manner characteristic of the Biverticil-

lata-Symmetrica section to which the species is assigned (*cf.* FIG. 11C); conidiophores borne primarily as branches from aerial hyphae; commonly $100\ \mu$ or less in length by 2.0 to $2.5\ \mu$, with walls smooth or irregularly roughened and colored in light yellow-green shades; metulae variable, when present commonly 10 to $15\ \mu$ by about $2.0\ \mu$, rarely occurring in clusters of more than 3 or 4; sterigmata tapered in the manner characteristic of the section, produced in limited clusters up to 6 or 7, variable in dimensions, 8 to $12\ \mu$ by 2.0 to $2.5\ \mu$, bearing conidia in divergent chains; conidia elliptical, smooth, about 3.0 to $3.5\ \mu$ by $2.5\ \mu$.

Colonies on malt extract agar spreading broadly, up to 6 to 7 cm. in twelve to fourteen days, plane (FIG. 3A), comparatively thin, consisting of a loose network of aerial mycelium in which abundant perithecia soon develop with accompanying encrusted and pigmented hyphae to give the colony a rich golden yellow color, in age near light cadmium to aniline yellow (Ridgway, Pl. IV); conidial structures lacking or limited in number, odor lacking; no exudate; reverse in brownish orange shades; perithecia generally spherical or nearly so (FIG. 3C), ranging from 100 to $300\ \mu$ in diameter, usually about 200 to $250\ \mu$, soft, loose-textured, sometimes confluent, without specialized cellular walls but bounded by thin networks of interwoven hyphae, and surrounded by loose coverings of twisted or spiral, encrusted and pigmented hyphae up to 150 to $200\ \mu$ or more in length; asci produced abundantly throughout, borne in short chains, at maturity spherical to ovate, 5.5 to $7.0\ \mu$ in diameter, 8-spored, walls of asci breaking down quickly to leave the perithecial cavity filled with free ascospores; ascospores very small, elliptical (FIG. 3D), about 2.5 to $3.0\ \mu$ by 1.4 to $1.8\ \mu$, delicately spinulose over the entire surface.

Colonies on corn meal agar spreading fairly broadly, 5 to 6 cm. in two weeks, vegetative mycelium largely submerged and producing scattered perithecia and limited conidial structures, mostly fractional.

Perithecial initials (FIG. 3B) readily observed upon most substrata, particularly upon corn meal agar. These first appear as thickened, clubshaped hyphae (ascogonia?) around the base of which coil much thinner hyphae (antheridia?). The latter type of hypha confines itself to the basal area of the club-shaped structure and usually terminates as a slight enlargement closely appressed against the wall of the larger hypha. The club-shaped hypha apparently elongates and soon begins to coil terminally, at first in a loose helix-like pattern and subsequently as a rather tight spiral. As the spiral continues to develop, its identity is soon lost in a developing knot of interwoven tissue. We have not succeeded in establishing whether this knot develops primarily by the septation

and repeated branching of the coiled structure or by the proliferation of adjacent hyphae. The developmental history of the perithecium has not been elucidated, but the origin and pattern of ascus formation can be fairly well worked out in the ripening perithecium.

Species description based upon NRRL 2106 as **type**, isolated from soil sent to us in 1945 from Sweden by Professor Edy Velander.

Penicillium helicum is distinguished from other members of the ascosporic *P. luteum* series by the coiled helix-like pattern of its perithecial initials and the small dimensions of its ascospores. When young the perithecial initials are strongly suggestive of *P. vermiculatum* Dangeard (1907), but as these develop they assume a markedly different and specific pattern.

The name, *Penicillium helicum*, is based upon the characteristically coiled structures that distinguish the species, and is taken from the Latin *helica*, meaning winding.

***Penicillium rotundum* sp. nov.**

Coloniae in agaris omnibus restrictae, satis compactae, primum plurimum mycelicae sed mox perithecia abundantia gerentes, aureo-flavae, reverso aurantio vel rubro; fructificatione conidica sparsa; conidiophoris e substrato orientibus, usque $200 \times 3.0 \mu$; penicillis typice biverticillatis et symmetricis, sed saepe non ab omni parte; metulis in verticillis 4 vel paucioribus, $10-13 \times 2.0-2.5 \mu$; sterigmatibus lanceolatis, apicibus acuminatis, strictim parallelibus, in 4-7 verticillis, circa $10-12 \times 1.5-2.0 \mu$; conidiis ellipticis, $2.5-3.0 \times 2.0-2.5 \mu$ glabris; peritheciis globosis vel subglobosis, $150-300 \mu$ in diam., laxe textis, mollibus, rete hypharum intertextarum limitatis, interdum confluentibus, cito maturescentibus; ascis catenatis, ovatis vel globosis, $12.5-15.0 \mu$ in diam., octosporis; ascosporis globosis, crasse tunicatis, superficiem totam echinulatis, $4.5-5.0 \mu$ in diam.

In culturis e ligno, Panama.

Colonies on Czapek's solution agar growing very restrictedly, about 1.0 cm. in two weeks at room temperature, consisting of a compact, fairly tough felt up to 500μ or more deep, at first largely mycelial but developing abundant inconspicuous perithecia (see malt agar) after one week to ten days and showing bright golden yellow shades from pigmentation of the perithecia and the encrusted hyphae surrounding them; penicilli generally lacking or, if present, limited in number and usually fragmentary (see 20 per cent sucrose Czapek), not affecting the colony appearance; exudate limited, light amber in color; odor slight or lacking; re-

verse in yellow to orange-red shades with surrounding agar lightly colored.

Colonies on Czapek's solution agar with 20 per cent sucrose growing restrictedly as above, plane, producing abundant conidial structures in a dense stand, velvety or nearly so, in pale gray-green shades near olive gray (Ridgway, Pl. LI) to storm gray (R., Pl. LII), conidiophores arising primarily from the substratum, com-

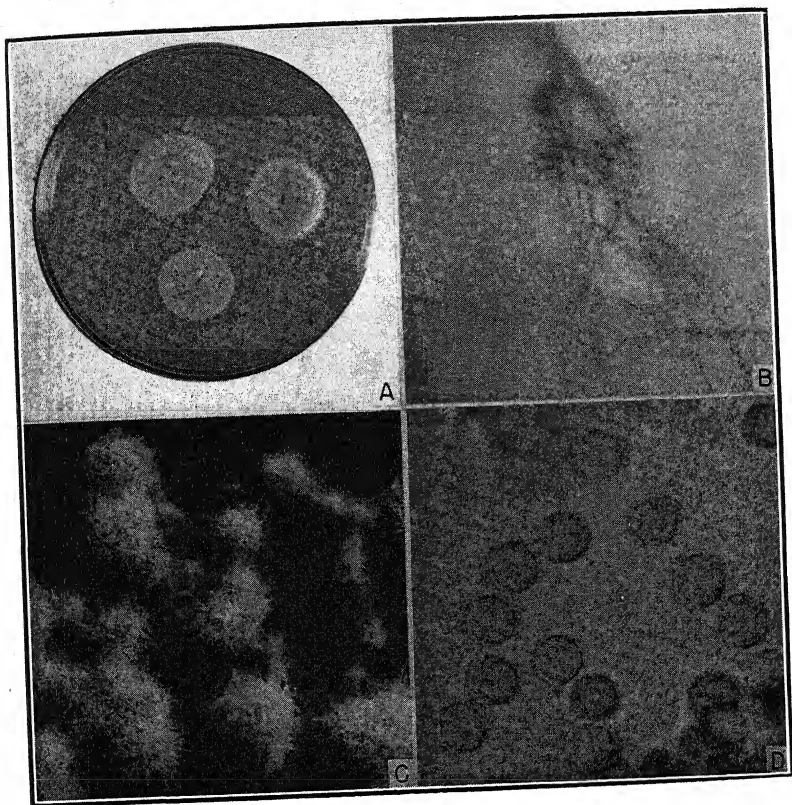


FIG. 4. *Penicillium rotundum*.

paratively short, seldom exceeding $200\ \mu$ in length by $3.0\ \mu$ in diameter; penicilli variable, ranging from fractional or monoverticillate to typically biverticillate and symmetrical (cf. FIG. 11C); metulae in limited verticils, rarely exceeding 4 in number, 10 to $13\ \mu$ by 2.0 to $2.5\ \mu$; sterigmata closely parallel, in clusters of 4 to 6 or 7, about 10 to $12\ \mu$ by 1.5 to $2.0\ \mu$, with conidium-bearing tips definitely tapered in the manner characteristic of the Biverticillata-Symmetrica section to which the species unmistakably belongs; conidia

elliptical, with ends more or less pointed, mostly 2.5 to 3.0 μ by 2.0 to 2.5 μ , smooth-walled.

Colonies on malt extract agar growing slowly (FIG. 4A), 1.5 to 2.0 cm. in two weeks, in bright golden yellow shades near lemon-chrome to light cadmium (Ridgway, Pl. IV), with growing margin 1 to 2 mm. wide, thin, often largely submerged, quickly developing abundant perithecia to form a continuous layer which in the main constitutes the colony, becoming rich golden orange near deep chrome to cadmium yellow (R., Pl. III) after two weeks; perithecia usually globose or nearly so but varying greatly in size from 150 to 300 or 350 μ in diameter, sometimes confluent, without definite cellular walls, bounded by a thin network of interwoven hyphae (FIG. 4C) and surrounded by a loose covering of predominantly radiate, heavily encrusted, and strongly pigmented hyphae, 100 μ or more in length; perithecia ripening within 7 to 10 days, producing abundant asci throughout; asci borne in short chains, ovate to globose when mature, 12.5 to 15.0 μ in diameter, 8-spored; ascospores globose (FIG. 4D), definitely echinulate over the entire surface, mostly 4.5 to 5.0 μ in diameter, with walls heavy, 1.0 μ or more thick.

Colonies on corn meal agar slow-growing, about 2 cm. in 2 weeks, thin with mycelium largely submerged, producing perithecia abundantly at colony center and scattered throughout the entire colony area, in form and development as on malt; penicilli very limited in number.

Perithecial initials (FIG. 4B) readily observed at the margin of growing colonies upon moist substrata, particularly corn meal and malt extract agars, irregular in origin and pattern (not consistent as in *Penicillium vermiculatum* Dangeard and *P. helicum*), first evident as swollen and irregularly septate hyphal elements which may be more or less twisted or coiled and which may arise either directly from vegetative hyphae, or from structures at first appearing penicillate, quickly developing into a knot of twisted and interwoven hyphal elements. Definite ascogonia are usually not identifiable.

Species description based upon NRRL 2107 as **type**, received in March 1946 from Professor G. W. Martin, University of Iowa, as an isolate from wood collected in the mountains of Chiriqui Province, Panama.

The species is distinguished by its restricted growth upon all substrata, the rich golden yellow color of its massed perithecia, the variability of its perithecial initials, and particularly by its large

globose ascospores. The specific name is based upon the shape of the ascospores.

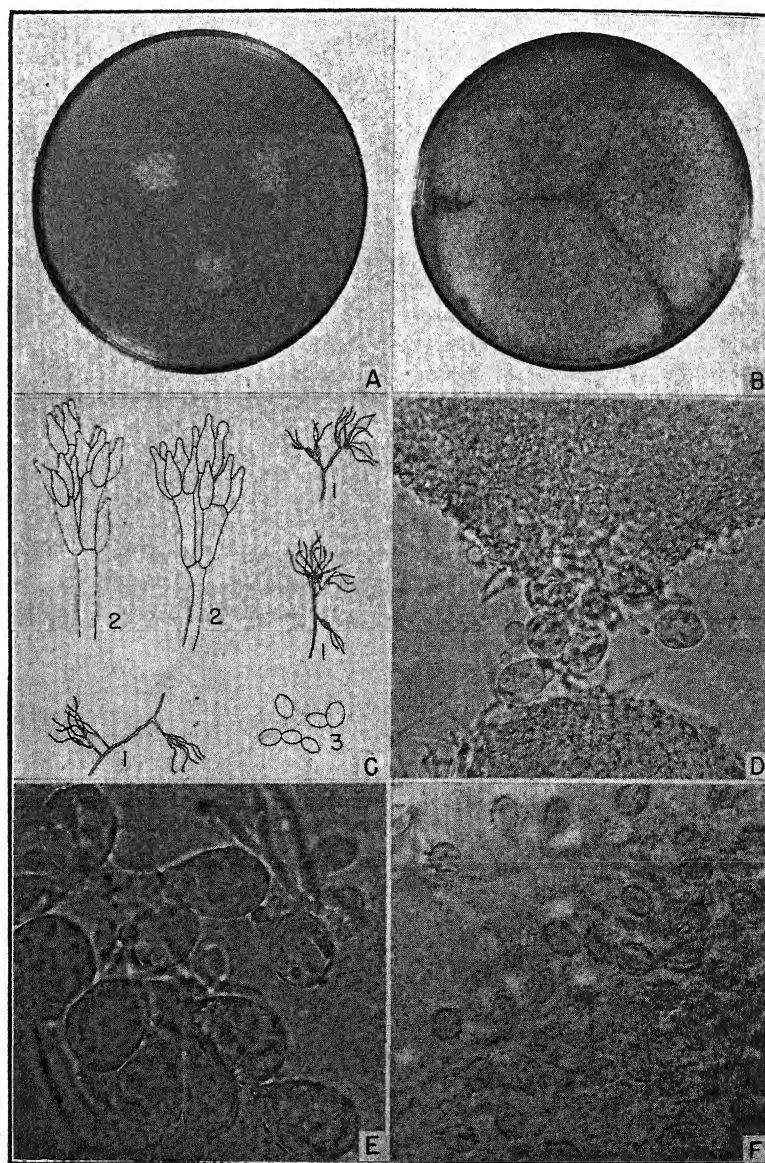
The species is believed to be more closely related to *Penicillium wortmanni* Klöcker (1909) than to other members of the ascosporic *P. luteum* series. It differs from *P. wortmanni* principally in the character of its perithecial initials and its globose ascospores. In form, the latter are similar to the spores of *P. bacillosporium* Swift (1932) but are consistently larger. It is readily distinguished from the latter species by differences in habits of growth, coloration, and particularly conidial patterns—the conidia of Swift's species being strongly bacilliform as the name implies.

Penicillium striatum sp. nov.

Coloniae in agar Czapekii restrictae sed in maltoso late crescentes, comparative tenues, penicillos paucos sed perithecia abundantia gerentes, albae usque pallide bubulinae, reverso rubro-brunneo vel purpurascenti; conidiophoris ex hyphis aereis orientibus, glabris; penicillis parvis, plerumque monoverticillatis, interdum biverticillatis sed asymmetricalis; metulis si praesentibus altitudinibus differentibus orientibus, $8.0-10.0 \times 3.0-3.5 \mu$; sterigmatibus in quoque verticillo paucis, $8.0-10.0 \times 2.5-3.0 \mu$, apicibus distincte attenuatis; conidiis mox deciduis, ellipticalibus, $3.0-4.0 \times 2.5-3.0 \mu$, glabris; peritheciis laxae textis, byssoideis peridio indefinito, usque 150μ diam.; ascis singulis, oblongis usque sphaericalibus, circa 15μ diam.; ascosporis ellipticis, $7.0-8.5 \times 5.0-6.0 \mu$, 8-10 taeniolis undulatis, latis, longitudinalibus, apices sporaе versus convergentibus cinctis.

In culturis e fructo *Vaccinii* condito.

Colonies on Czapek's solution agar growing very restrictedly (FIG. 5A), attaining a diameter of 1.0 to 1.5 cm. in two weeks at room temperature, with margin uneven from the irregular and localized growth of the vegetative mycelium, growing deeply in the agar with aerial hyphae and later perithecia developing above this deep mycelial growth, white to pale buff in color, with surface mealy or granular, conidial structures very limited in number and not affecting the colony appearance, vegetative mycelium comparatively coarse with hyphal tips at colony margin often showing inflated cells; perithecia abundantly produced, developing throughout the entire area, loose-textured, cottony, without definite cellular walls (FIG. 5D), ranging up to 100 to 150μ in diameter; exudate not produced; odor lacking or indefinite; reverse at first colorless becoming dull brown in age; penicilli very sparsely produced (see 20 per cent sucrose Czapek). Perithecia ripening rather quickly with asci containing immature spores within seven to eight

FIG. 5. *Penicillium striatum*.

days and with ripe ascospores present in twelve to fourteen days; asci apparently arising as branches from fertile hyphae, not in chains (FIG. 5E), oblong to spherical, about $15\ \mu$ in diameter, 8-spored; ascospores comparatively large, elliptical, with over-all dimensions ranging from 7.0 to 8.5 by 5.0 to 6.0 μ , with walls bearing a series of wavy, longitudinal flanges or frills (FIG. 5F), about 1.0 μ in width, usually extending the entire length of the spore, and tending to converge at the two ends.

Colonies on steep agar growing more rapidly, attaining a diameter of 3.5 to 4.5 cm. in two weeks at room temperature, with margins more regular than above, often entire, with surface appearing slightly granular from abundant perithecia or almost velvety where these become confluent, at first white, becoming buff to light brown in central areas in age; exudate very limited, clear; reverse in dull to reddish brown shades; penicilli very limited in number; perithecia very abundant, often forming a continuous layer; ascospore development and measurement as above.

Colonies on malt agar spreading broadly, attaining a diameter of 6.0 to 7.0 cm. in two weeks, thin, plane (FIG. 5B), conspicuously granular in appearance with perithecia in a dense layer at the agar surface and surrounded by thin loose networks of vegetative hyphae; reverse ranging from dull brown to purple; penicilli sparsely produced; perithecia ripening within ten days.

Colonies on 20 percent sucrose Czapek agar thin, restricted, producing penicilli more abundantly than on the above media; penicilli irregular in pattern and complexity (FIG. 5C), commonly monoverticillate but often developing as biverticillate structures without consistent arrangement of parts (FIG. 5C₂); conidiophores mostly short, 50 μ or less, borne as branches from aerial hyphae (FIG. 5C₁), occasionally arising from the substratum and 100 μ or more in length, smooth-walled, about 3.0 μ in diameter; penicilli simple, monoverticillate and consisting of a terminal verticil of 3 to 5 or 6 sterigmata, or biverticillate with 2 or more metulae or branches arising at a single or different levels (FIG. 5C), 8 to 10 μ or more in length by 3.0 to 3.5 μ in diameter, occasionally almost ramigenous and consisting of a number of short, divergent, irregularly arranged, branches bearing sterigmata; sterigmata variable in form and dimensions but mostly 8 to 10 μ by 2.5 to 3.0 μ with conidium-bearing tips short and definitely narrowed; conidia quickly deciduous, elliptical (FIG. 5C₃), mostly 3.0 to 4.0 μ by 2.5 to 3.0 μ , with ends usually somewhat pointed, walls smooth and comparatively heavy.

Species description centered upon NRRL 717, as **type**, isolated in 1938 by Williams, Cameron, and Williams (1941) from canned

blueberries. A second strain, NRRL 2080, was isolated in January 1946 from a sample of soil sent to us from Sweden by Professor Edy Velander. This latter culture differs from the former only in producing colonies slightly more restricted and ascospores with flanges less consistently parallel. A third strain similar to the second was isolated from Swedish soil but was not retained.

The proper placement of *Penicillium striatum* remains in doubt. The general characteristics of its perithecia are somewhat suggestive of the genus *Gymnoascus*. Among the ascosporic *Penicillia*, they are most nearly approximated in *P. wortmanni* Klöcker, *P. luteum* Zukal (1889), and allied members of the *P. luteum* series. Saccardo (Sylloge XI, p. 437. 1895) went so far as to transfer Zukal's species to Baranetzky's genus *Gymnoascus*. The ascospores are unusually large, and show a unique type of ornamentation. Conidial structures vary in complexity from strictly monoverticillate, composed of terminal verticils of sterigmata numbering up to 5 or 6 or occasionally more, to variously branched and biverticillate, but never show either the characteristic symmetric pattern or the long tapered sterigmata that are characteristic of the Biverticillata-Symmetrica section. Despite the basic differences shown by its conidial stage, the species is tentatively placed in the *P. luteum* series with other species producing soft, loose-textured perithecia without walls composed of definitely specialized cells. More exact placement must await further examination of the developmental history of the fungus, or the isolation of additional strains transitional between it and other well defined species.

NON-ASCOSPORIC SPECIES

Sclerotigenic Species

Penicillium lapidosum sp. nov.

Coloniae expandentes, comparative tenues, planae vel subplanae, mycelio vegeto largiter submerso, sclerotiis mox crescentibus, abundantibus, primum aurantiacae demum aurantio-brunneae vel rubescentes, reverso concolori; fructificatione conidica sparsa; exsudato plerumque abundanti, aurantiorubro; conidiophoris sparsis, ex hyphis repentibus orientibus, plerumque $25-75 \times 2.5-3.5 \mu$; penicillis variabilibus, plurimum monoverticillatis sed interdum 1-2-ramosis; sterigmatibus in verticillis 7-8 compactis, $6.0-7.5 \times$ circa 2.0μ , apicibus attenuatis; conidiis ellipticis vel interdum subglobosis, $2.5-3.0 \times 2.0-$

2.5 μ , glabris, in catenis laxae parallelibus usque 100 μ longis; sclerotiis, globosis vel subglobosis, usque 300–350 μ in diam., perduris, lapidosus, e cellulis crasse tunicatis 10–15 μ in diam. compositis.

In culturis e fructo *Vaccinii* condito, Washington, D. C.

Colonies on Czapek's solution agar spreading broadly, attaining a diameter of 5.0 to 6.0 cm. within two weeks at room temperature, plane (FIG. 6A) or lightly furrowed, golden orange in color, de-

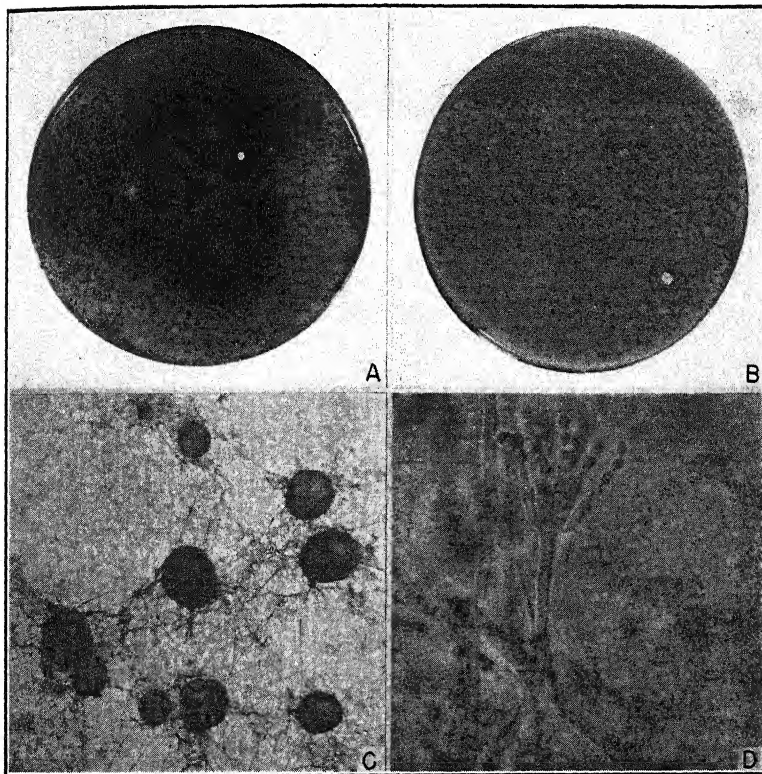


FIG. 6. *Penicillium lapidosum*.

veloping reddish tints in age, consisting of an extensive vegetative mycelium largely submerged, developing abundant orange-brown sclerotia in a fairly dense layer on the agar surface, with limited development of sterile aerial hyphae often more or less obscuring the individual sclerotia; penicilli rarely produced and not affecting the colony appearance (see hay agar below); exudate abundant, in orange-red shades; odor lacking or indistinct; reverse in orange-red shades becoming deep reddish brown in age; sclerotia globose to

subglobose, variable in size up to 300 to 350 μ in diameter (FIG. 6C), very hard, stony, crushing with difficulty, composed of very thick-walled polygonal cells mostly 10 to 15 μ in diameter.

Colonies on steep agar as on Czapek but growing even more rapidly, generally producing more abundant exudate, and more intensely colored reverse; penicilli very sparsely produced; sclerotia as above.

Colonies on malt agar spreading broadly, plane (FIG. 6B), quickly developing golden yellow shades from abundant orange colored sclerotia and enveloping yellow encrusted sterile hyphae; exudate abundant, clear; penicilli developing fairly abundantly in older colony areas but not affecting the overall appearance of the culture.

Colonies on hay infusion agar growing rapidly, thin, consisting of a spreading, submerged vegetative mycelium, producing sclerotia in limited numbers in a thin layer on the agar surface, numerous penicilli borne on short lateral branches or conidiophores from trailing hyphae; sclerotia as described above but generally smaller, rarely exceeding 200 μ in diameter; penicilli variable, mostly strictly monoverticillate (FIG. 6D), consisting of compact verticils of sterigmata bearing tangled or loosely parallel chains of conidia up to 100 μ in length, occasional penicilli once or twice branched and producing two or more clusters of sterigmata; conidiophores mostly 25 to 75 μ , rarely more than 100 μ in length by 2.5 to 3.0 or 3.5 μ in diameter, with walls smooth, commonly septate; branches, when present, mostly 10 to 20 μ by 2.5 to 3.0 μ , more or less divergent; sterigmata ranging from 3 or 4 up to 7 or 8 in the verticil, mostly 6.0 to 7.5 μ by about 2.0 μ with conidium-bearing tips definitely narrowed (FIG. 6D); conidia at first definitely elliptical, usually remaining so and ranging from 2.5 to 3.0 μ by 2.0 to 2.5 μ , occasionally subglobose, 2.0 to 2.5 μ in diameter, with walls smooth and comparatively heavy.

Species description based upon NRRL 718 as **type**, isolated in 1938 from canned blueberries by Dr. E. J. Cameron and associates, National Canners Association, Washington, D. C.

Williams, Cameron, and Williams (1941) reported the sclerotia of this mold to be unusually heat-tolerant, being able to withstand a temperature of 90.5° C. for thirty to forty minutes. The culture also was reported to be able to grow (or survive) in a high vacuum. These investigators reported the successful isolation of the mold from three of five soil samples collected from blueberry fields and

heated in the laboratory to 180° F. for twenty-five minutes. They failed, however, to distinguish between the form which produced ascospores (*Penicillium striatum*) and that which produced sclerotia—in fact, they apparently regarded the two strains as representing different aspects of the same fungus. Their report failed to state whether both types, sclerotial and ascosporic, were re-isolated from soil or whether the sclerotial form only was so obtained.

The type strain of *Penicillium lapidosum* is characterized particularly by the abundant sclerotia which it produces. In appearance and texture, these are strongly suggestive of the young, sclerotoid perithecia which characterize certain ascosporic species such as *P. parvum* and *P. baarnense* van Beyma (1939–1940). At no time, however, have we observed any evidence of ascospore formation in this strain—the bodies remaining hard and sclerotoid indefinitely. The species is assigned to the sclerotigenic series of monoverticillate *Penicillia* with *P. thomii* Maire (1917).

An additional culture, essentially duplicating NRRL 718, isolated at Baarn in 1939, was received from the Centraalbureau in June 1946 as *Penicillium mangini* Duché and Heim. This is now maintained in our collection as NRRL 2084. The above cultures seem to agree reasonably well with Duché and Heim's description, in both the character of the sclerotia produced and the manner in which the penicilli are borne on trailing aerial hyphae. Furthermore, the penicilli are not consistently monoverticillate but frequently produce branched structures of the type illustrated by Duché and Heim (1931). Were it not for the fact that Duché, in personal conference with us in our Laboratory and with the cultures in question before him (January, 1947), indicated that his species was originally based upon a different type of organism, we would have concluded that NRRL 718 and 2084 accurately represented *P. mangini*. However, since these forms apparently do not represent his species, and since they cannot be satisfactorily assigned to any other described form, we recognize them as representing a new species to which the binomial *P. lapidosum* is assigned because of the abundant stone-like sclerotia that characterize it.

*Conidial Species****Penicillium capsulatum* sp. nov.**

Coloniae in agaris omnibus restrictae, e coacta tenui mycelica superficie velutina vel floccosa constitutae, saepe profunde furcatae vel rugosae, sporulatione media, primum pallide griseo-virides, in culturis senibus fusciscentibus; exudato odoreque carentibus; conidiophoris ascendentibus, plerumque ex hyphis repentibus orientibus, brevissimis vel usque $100 \times 2.0-2.5 \mu$, glabris, irregulariter ramosis; penicillis monoverticillatis, interdum caespitosis, sed distinctis; sterigmatibus in 5-10 verticillis simplicibus, compactis dispositis, plerumque parallelis, $8.0-10.0 \times 2.0-2.2 \mu$; conidiis ellipticis usque capsulatis, $3.0-4.0 \times 2.0-2.5 \mu$, glabris, in columnis usque 100μ longis vel longioribus catenatis.

In culturis ex instrumento opticali, Panama Canal Zone.

Colonies on Czapek's solution agar growing restrictedly, attaining a diameter of 2.0 to 2.5 cm. in twelve to fourteen days at room temperature, consisting of a comparatively thin, close-textured mycelial felt, tough, tearing irregularly, with surface appearing velvety or very slightly floccose, deeply furrowed (FIG. 7A), commonly raised or depressed in central area, azonate in most strains, with growing margin narrow, about 1 mm. wide, white, medium sporing throughout, in gray-green shades, at first approximately court gray or gnaphalium green (Ridgway, Pl. XLVII) becoming darker in age near sage green (R., Pl. XLVII), exudate lacking; odor lacking or indefinite; reverse uncolored or slightly greenish at first but later showing orange to pinkish shades; conidiophores ascending, arising primarily from creeping or interlacing hyphae (FIG. 7C), from very short up to 100μ or more in length by 2.0 to 2.5μ in diameter, walls smooth, branching irregularly, occasionally over their entire length, but more abundantly in terminal areas; penicilli monoverticillate, borne on branches of varying length and occasionally more or less clustered but consistently retaining their individual character (FIG. 7D); sterigmata borne irregularly but typically in simple clusters, ranging from 4 or 5 up to 8 or 10 in the verticil, usually crowded, parallel, sometimes divergent, mostly 8 to 10μ by 2.0 to 2.2μ but not infrequently larger or smaller; conidia strongly elliptical, commonly capsule-shaped, mostly 3.0 to 4.0μ by 2.0 to 2.5μ , but frequently larger, with walls smooth.

Colonies on steep agar growing more rapidly, 2.5 to 3.0 cm. in ten to twelve days, texture as described above but more strongly and deeply furrowed, heavier sporing throughout, at first near pea green becoming sage green in colony center (R., Pl. XLVII); exudate limited, pale yellow; odor sourish; reverse uncolored or

in yellow shades; conidiophores and penicilli as described above; conidia more consistently capsule-shaped.

Colonies on malt agar about 2.5 to 3.0 cm. in ten to twelve days, comparatively thin with center commonly raised, velvety, heavily sporing throughout (FIG. 7B), pea green to sage green, reverse in dull yellow to drab shades; conidiophores longer, up to 200 to 300 μ and loosely branched, commonly arising from the substratum, with

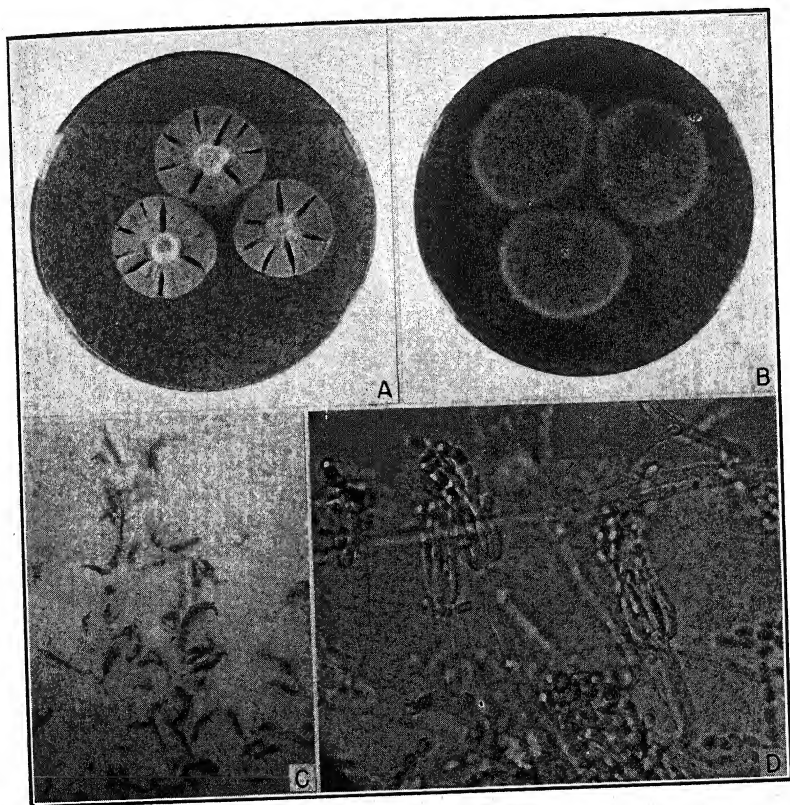


FIG. 7. *Penicillium capsulatum*.

walls smooth but appearing granular within; penicilli more regular in pattern than on Czapek or steep agar, usually consisting of a closely crowded cluster of 6 to 12 or more parallel sterigmata, 7 to 8 μ by 1.5 to 2.0 μ , bearing conidia in long parallel chains forming compact columns, about 10 μ in diameter and up to 150 μ or more in length; conidia very strongly elliptical to narrowly cylindrical, mostly 3.0 to 4.0 μ by 1.5 to 2.0 μ , occasionally up to 6.0 to 7.0 μ

by 2.0 to 3.0 μ , with walls smooth, adhering in long chains when viewed in fluid mounts.

The binomial *Penicillium capsulatum* is assigned to the species because of the characteristic strongly elliptical to narrowly cylindrical form of its conidia which duplicate in pattern the gelatin capsules used in pharmaceutical trade.

Species description centered upon NRRL 2056 as **type**, received in September 1945, from Professor W. H. Weston, Harvard University, as a culture isolated in the Panama Canal Zone from an optical instrument by Dr. W. G. Hutchinson. It is duplicated also by NRRL 2057, received in May 1945, from Dr. W. Lawrence White, Philadelphia Quartermaster Depot, as a strain isolated from exposed canvas in the Gilbert Islands.

Additional strains representing this species have been repeatedly encountered among the molds isolated from deteriorating military equipment in tropical and sub-tropical areas. No information is available regarding the extent of growth or the amount of damage caused by this organism, but its repeated isolation from such sources would seem to indicate its probable wide distribution in , tropical and sub-tropical areas.

***Penicillium lavendulum* sp. nov.**

Coloniae in agaro Czapekii late effusae, comparative tenues, mycelio vegeto plurimum submerso, superficiali laxo vel flocculento et funiculoso; fructificatione in areas centrales densa, griseo-lavendula, margine incolorata, in agaro maltoso abundanti, vinacea; reverso purpureo-vinaceo; conidiophoris e substrato vel hyphis repentibus orientibus, fere 100–150 \times 3.0–3.5 μ , conspicue echinulatis; penicillis asymmetricis infra metulas 1–2 ramosis, ramis variabilibus, usque 15–20 \times 2.5–3.0 μ , valde echinulatis; metulis 8.0–10.0 \times 2.0–3.0 μ , plerumque echinulatis; sterigmatibus strictim parallelibus, dense confertis, 7.0–9.0 \times 2.0–2.2 μ , saepe asperatis; conidiis ellipticis, variabilibus, 3.0–4.5 \times 2.0–3.0 μ , glabris, in catenis longis saepe laxe parallelibus.

Contaminatione in laboratorio, Peoria, Illinois.

Colonies on Czapek's solution agar spreading broadly, attaining a diameter of 5.0 to 6.0 cm. in twelve to fourteen days at room temperature, plane or nearly so, azonate, comparatively thin with vegetative mycelium largely submerged and with surface growth consisting of a loose web of flocculent to cottony hyphae, showing some ropiness mostly in marginal to submarginal areas, sporulating abundantly in central areas (FIG. 8A), approximating dark

grayish lavender to Ramier blue (Ridgway, Pl. XLIII), thinning through lighter shades to uncolored at the colony margin; exudate limited, in small drops, colorless; odor slight; reverse uncolored to light purple; penicilli variable in size, with conidial chains up to $100\ \mu$ in length, loosely parallel, tangled or matted (FIG. 8C); co-

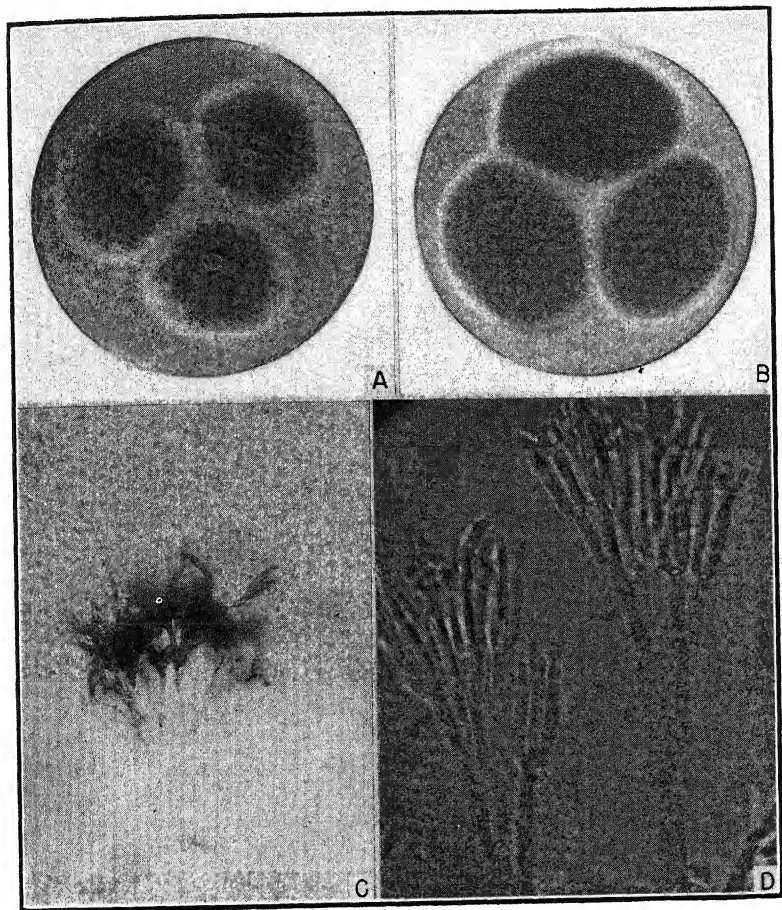


FIG. 8. *Penicillium lavendulum*.

nidiophores sometimes arising from the substratum, but borne primarily as branches from aerial hyphae, commonly 100 to $150\ \mu$ in length by 3.0 to $3.5\ \mu$ in diameter, sometimes shorter, septate, with walls closely echinulate; penicilli asymmetrical, irregularly once- or twice-branched below the metulae, with metulae commonly arising at different levels within the penicillus; branches mostly

2.5 to 3.0 μ in diameter, varying greatly in length up to 15 to 20 μ , rough; metulae mostly 8 to 10 μ by 2.5 to 3.0 μ , usually roughened (FIG. 8D); sterigmata in compact clusters, closely parallel, 7 to 9 μ by 2.0 to 2.2 μ , with apices slightly narrowed but lacking well-defined conidium-bearing tubes, commonly rough-walled; conidia narrowly elliptical (FIG. 8D), from 3.0 to 4.5 μ by 2.0 to 3.0 μ , with walls smooth and comparatively heavy, tending to adhere in chains in fluid mounts.

Colonies on steep agar growing as on Czapek but somewhat deeper and generally heavier sporing, predominantly dark grayish lavender but often appearing somewhat mottled from irregular spore production in submarginal areas; reverse in purple-vinaceous shades, thinning toward the margin; penicilli as described above but commonly larger, with cellular elements somewhat longer; conidia more narrowly elliptical, capsule-shaped.

Colonies on malt extract agar, broadly spreading, plane, like the preceding in pattern and texture but usually heavier sporing, with massed penicilli forming crusts of conidia up to 400 or 500 μ deep (FIG. 8B), developing reddish tints to form shades near deep purplish vinaceous to dull Indian purple (R., Pl. XLIV); reverse in dull to deep purple vinaceous shades; conidiophores arising mainly from the substratum; penicilli as described above but commonly larger and more complex, up to 50 or 60 μ in length, with walls of conidiophores, branches, and metulae conspicuously roughened; conidia narrowly elliptical or capsule-shaped, 4.0 to 4.5 μ by 2.0 to 2.5 μ , smooth-walled.

Species description based upon NRRL 2146 as **type**, isolated in July 1947 as a laboratory contaminant at the Northern Regional Research Laboratory.

The binomial *Penicillium lavendulum* is based upon the characteristic coloring of the species upon Czapek's solution and steep agars.

The correct placement of the species remains in doubt, but it appears to be most satisfactorily assignable to a series with *Penicillium pallidum* Smith (1933) in the Funiculosa subsection of the Asymmetrica. The conspicuously roughened conidiophores and cellular elements of the penicilli, its narrowly elliptical to capsule-shaped conidia, and the funiculose character of its colonies on Czapek's agar all tend to relate it to this series more closely than to any other recognized group. Furthermore, colonies on malt agar occasionally develop limited sectors or overgrowths character-

ized by almost colorless conidia, and isolations made from such areas commonly show little or no pigmentation of conidia. In gross appearance these substrains are often strongly suggestive of the species *P. pallidum*.

Bainier (1906) described a species, *Penicillium rubescens*, characterized by narrowly elliptical conidia with fruiting areas in reddish or rusty shades. Penicilli were described and figured as complex and repeatedly branched, with cellular elements coarse and very short. In *P. lavendulum*, however, cellular elements are quite long and thin and the penicilli commonly appear somewhat laterally appressed. Were it not for such marked differences in the general patterns of the penicilli of the two forms, our culture might possibly be regarded as representing Bainier's species.

Penicillium piceum sp. nov.

Coloniae in agar Czapekii tenues, mycelio vegeto plurimum submerso vel e coacta laxa aerea, satis funiculosa constitutae, fructificationes conidicas paucas vel abundantes gerentes, sordide flavo-virides, reverso rubro-aureo usque rubro-brunneo; guttulis microscopicis abundantibus; conidiophoris glabris, brevibus, rare $50 \times 2.5-3.0 \mu$ excedentibus, rete hypharum multo ramosarum orientibus; penicillis dense compactis, biverticillatis et symmetricis, metulis 10-12, verticillis densis sterigmatium et catenarum conidicarum compositis et massam conicam vel pyramidalem usque 150μ longam efformantibus; metulis $8-10 \times 1.8-2.2 \mu$, exterioribus incurvatis; sterigmatibus lanceolatis apicibus acuminatis, $8-9 \times 1.5-1.8 \mu$, parallelibus, catenas conidiorum strictim adherentes gerentibus; conidiis subglobosis vel ellipticis, $2.5-3.0 \times 2.2-2.8 \mu$, primum glabris, saepe in aetate irregulariter asperatis.

In cultura indeterminata, Thom Collection.

Colonies on Czapek's solution agar growing somewhat restrictedly (FIG. 9A), attaining a diameter of 3.0 to 3.5 cm. in twelve to fourteen days at room temperature, often with central area 1.0 to 1.5 cm. raised, consisting primarily of a thin, white to yellow mycelial felt bearing few conidial heads but surrounded by a thinner plane marginal zone producing abundant conidial structures in a loose mycelial network, in dull yellow-green shades near tea green or pea green (Ridgway, Pl. XLVII), occasional strains lacking the prominent thin margin, but showing the entire colony area felted, producing limited numbers of conidial structures and considerable sterile mycelium throughout; exudate fairly abundant, mostly as microscopic droplets adherent to the mycelium; odor distinct, rather pleasant; reverse in brownish orange shades, with the green of conidial areas showing through in marginal areas; conidiophores

short (FIG. 9D), arising from a loose network of much-branched aerial hyphae, usually about 20 to 35 μ by 2.5 to 3.0 μ , rarely 50 μ or more, in length, smooth-walled; penicilli very compact, typically biverticillate-symmetrical (FIG. 9E), consisting of 10 to 12 metulae borne on the vesicular apices of the conidiophores; metulae 8 to 10 μ by 1.8 to 2.2 μ , with outer metulae incurved, tending to become parallel with the main axis of the conidiophore; sterigmata

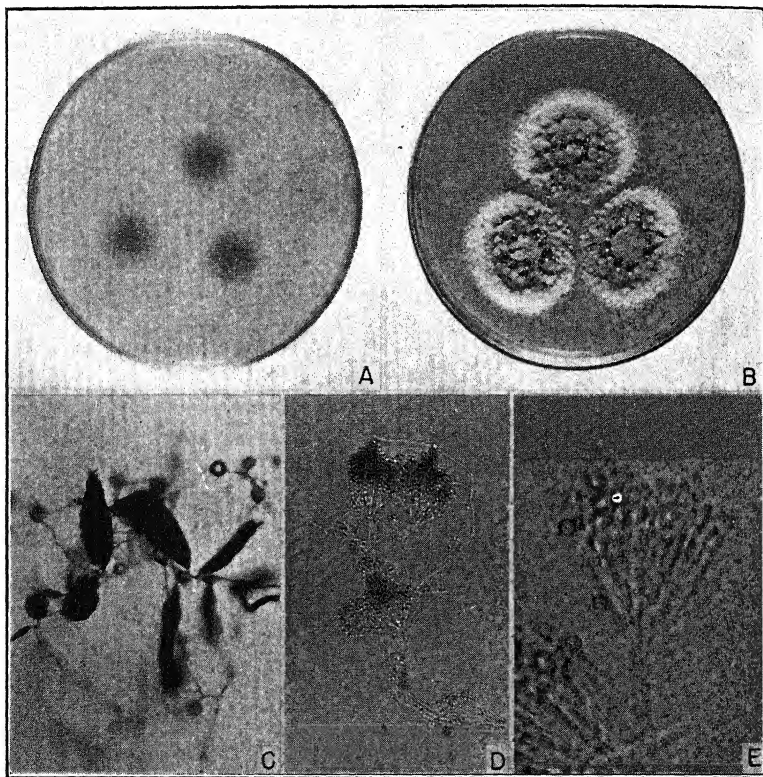


FIG. 9. *Penicillium piceum*.

typical of the Biverticillata-Symmetrica, lanceolate with apices acuminate, parallel, occurring in crowded clusters of 5 to 7, 8.0 to 9.0 μ by 1.5 to 1.8 μ , producing conidia in closely adherent chains which consistently produce conical to pyramidal masses up to 150 μ long (FIG. 9C); conidia subglobose to elliptical, 2.5 to 3.0 μ by 2.2 to 2.8 μ , smooth when young, becoming heavy-walled and irregularly roughened when mature, dark olive-green in mass, chains adherent

and the masses of conidia retaining their conical shape even in liquid mounts.

Colonies on steep agar growing rapidly, 5.0 to 5.5 cm. in twelve to fourteen days at room temperature, plane, velvety in appearance but consisting of a loose network of dwarfed aerial hyphae, heavily sporing throughout in dull yellow-green shades near slate-olive (R., Pl. XLVII), surface of colonies overgrown to a greater or lesser degree by sterile encrusted yellow mycelium becoming conspicuous in some strains; exudate limited to abundant, in small droplets, clear to very light yellow; odor as on Czapek; reverse in orange or red-brown shades becoming almost black in older areas; details of penicilli as described in Czapek, but with masses of conidia less conspicuously cone-shaped and up to 200 μ in length.

Colonies on malt extract agar 4.5 to 5.0 cm. in diameter in twelve to fourteen days (FIG. 9B), 1 to 2 mm. deep, surface uneven from irregular tufts of encrusted funiculose hyphae; fairly heavily sporing, in yellow-green shades near vetiver to andover green (R., Pl. XLVII); exudate limited in amount, evaporating to leave pits in the colony surface; odor fragrant, faintly suggestive of apples; reverse uncolored or showing very slight orange tints; microscopic details as on Czapek.

Species description centered upon NRRL 1051 as **type**, from the Thom Collection as an unidentified culture. Duplicated by NRRL 1071 received in 1937 from C. W. Emmons, National Institute of Health, as an unidentified *Penicillium*; and NRRL 2112, received in 1945 from J. W. Groves, Ottawa, Canada, as an unidentified *Penicillium* isolated from alfalfa seed.

Careful consideration of all described species of *Penicillium* has failed to reveal one which adequately characterizes the above strains. We, therefore, regard the cultures in question as representing a new species, to which the binomial *Penicillium piceum* is applied because of the striking resemblance of the typical conidial head to a compact, spruce-like evergreen in miniature.

The species clearly belongs in the Biverticillata-Symmetrica section and is most satisfactorily assigned to the series with *Penicillium funiculosum* Thom (1910).

***Penicillium aculeatum* sp. nov.**

Coloniae in agaro Czapekii restrictae, coacta mycelica superficie velutina corrugata compositae, sporulatione media, flavido-viridae et saepe ex hyphis rubris supercretae, reverso vinaceo vel purpureo-rubro, in agaro maltoso late

crescentes, sporulatione densa, flavido-virides; conidiophoris e substrato vel coacta mycelica orientibus, 50–200 vel longioribus \times 3.0–4.0 μ , parietibus granulosis vel conspicue asperatis; penicillis comparative brevibus, typice biverticillatis et symmetricis, interdum non ab omni parte; metulis inflatis, 8.0–12.0 \times 4.5–5.5 μ ; sterigmatibus 7.0–9.0 \times 3.0–3.5 μ , saepe tumidis, apicibus acuminatis; conidiis globosis vel subglobosis, 3.0–3.5 μ in diam., crasse tunicatis et conspicue echinulatis, in catenis laxae parallelibus vel implicatis usque 150 μ longis.

In culturis e textile ad aerem exposito, Florida.

Colonies on Czapek's solution agar growing restrictedly (FIG. 10A), about 2 cm. in twelve to fourteen days at room temperature, consisting of a tough basal felt 1 to 2 mm. deep, often buckled and wrinkled, sometimes irregular in outline, medium sporing and velvety in appearance in central colony areas, developing yellow-green shades near celandine to artemisia green (Ridgway, Pl. XLVII), often with a pinkish cast from a limited overgrowth of red-pigmented hyphae and embedded droplets of exudate; growing margins 2 to 3 mm. wide, white to slightly pink, often appearing somewhat tufted or funiculose; exudate abundant, almost uncolored to definitely vinaceous, occurring in small droplets and often becoming overgrown by conidial areas as these develop; odor almost lacking; reverse in vinaceous or purplish red shades approximating mineral red to dark mineral red (R., Pl. XXVII) in older areas, not strongly discoloring the surrounding agar; conidiophores arising primarily from the mycelial felt, short, commonly about 50 μ , rarely up to 100 μ by 3.5 to 4.0 μ , with walls appearing somewhat granular; penicilli typically biverticillate and symmetrical but with fractional or monoverticillate structures commonly produced; metulae 8 to 12 μ by 4.5 to 5.5 μ , usually appearing definitely inflated; sterigmata 7 to 9 μ by 3.0 to 3.5 μ , often appearing somewhat swollen; conidia globose to subglobose, 3.0 to 3.5 μ in diameter, with walls comparatively heavy and conspicuously echinulate (FIG. 10C), borne in loosely parallel or tangled chains 75 to 100 μ in length.

Colonies on steep agar growing somewhat restrictedly but more rapidly than on Czapek, radially furrowed with center somewhat raised, medium sporing throughout, in dull yellow-green colors as above but with reduced development of pink aerial hyphae and an almost complete absence of pink exudate, growing margin about 1 mm. wide, white; reverse usually in lighter shades than on Czapek; conidial structures generally intermediate in pattern between those developed on Czapek and on malt agar.

Colonies on malt agar spreading broadly, up to 5.5 to 6.0 cm. in twelve to fourteen days, plane (FIG. 10B), heavily sporing throughout, in dark yellow-green shades near Lincoln green to dusky

olive-green (R., Pl. XLI); conidiophores arising primarily from the substratum, less commonly from trailing or ascending hyphae, mostly about $200\ \mu$ long, but ranging from 100 to $300\ \mu$ or more by about 2.5 to $3.0\ \mu$ in diameter, with walls commonly roughened; penicilli comparatively short, consisting of a terminal verticil of metulae bearing clusters of somewhat divergent sterigmata, but

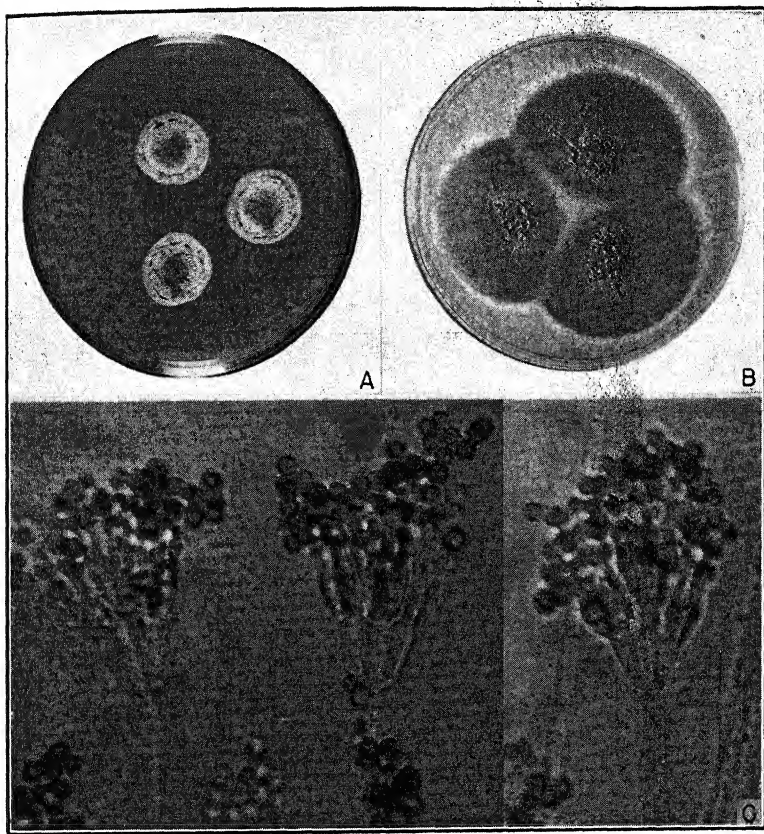


FIG. 10. *Penicillium aculeatum*.

withal typical of the Biverticillata-Symmetrica section of the genus (FIG. 10C); metulae occurring in verticils of 5 to 9, each bearing a crowded cluster of 5 to 7 sterigmata with finely granular walls and with strongly tapered conidium-bearing tips; conidia globose to subglobose, 3.0 to $3.5\ \mu$ in diameter, conspicuously echinulate, dark olive-green in mass, occurring in loosely parallel or tangled chains 100 to $150\ \mu$ in length.

Species description based upon a number of strains received from Professor William H. Weston, Harvard University, as unidentified cultures isolated from canvas and other materials infected during exposure tests in Florida. NRRL 2129 and NRRL 2130 (co-types) are representative. The species is regarded as new since careful examination of the literature has failed to reveal any described form which produces (1) globose and conspicuously rough conidia and (2) strongly roughened conidiophores in colonies with deep red colors in reverse.

The species name is taken from the Latin *aculeatus* (meaning "prickly"), and is applied because of the conspicuously echinulate character of conidia, and the roughened walls seen in conidiophores, metulae, and even sterigmata on malt extract agar where the species makes its maximum development.

Penicillium aculeatum is tentatively assigned to the *P. purpurogenum* series. Such placement is based upon two primary considerations: (1) colonies are more or less restricted on Czapek's solution and steep agars and produce a rich red to purple-red color in reverse, and (2) colonies on malt agar are broadly spreading, heavily sporing throughout, dark yellow-green in color, and essentially velvety. It differs from the other members of this series in producing conidial structures with walls often roughened and with conidia strongly echinulate and *globose* rather than elliptical to subglobose. Elements of the penicillus are usually shorter and tend to be divergent rather than closely parallel. In this latter characteristic the species resembles *P. verruculosum* Peyronel (1913), which also produces rough, globose conidia. The comparatively short, broad penicilli are suggestive of *P. herquei* Bainier and Sartory (1912) but the species shows little additional evidence of relationship in that direction. *Penicillium aculeatum* is separated from the *P. funiculosum* Thom (1910) and related species by an absence or limitation of funiculate hyphae. It is separated from *P. rugulosum* Thom (1910) by the production of abundant red to purple-red color in colony reverse, and by the character of its conidia. Despite the fact that the species does not conform too closely with other members of the *P. purpurogenum* series, it is our belief that workers encountering this species in culture will locate it here more conveniently than elsewhere.

Penicillium diversum sp. nov.

Coloniae in agar Czapekii neutro pertenuae et multo restrictae, in agar Czapekii acido et maltoso celeriter crescentes, luxuriosae, comparative tenues, planae, saepe anguste zonatae, sporulatione per omnes partes densa, griseae usque olivaceo-griseae; exudato carenti, reverso incolorato; conidiophoris e substrato orientibus, $200\text{--}300 \times 2.0\text{--}2.5 \mu$, glabris; penicillis biverticillatis et symmetricis, 5-8 metulis in verticillo terminali compositis; metulis $9\text{--}11 \times 2.0\text{--}2.5 \mu$, sursum leniter inflatis; sterigmatibus 6-8, dense caespitosis, lanceolatis, apicibus acuminatis, $8\text{--}10 \times 1.8\text{--}2.2 \mu$; conidiis ellipticis, $2.0\text{--}2.5 \times 1.5\text{--}2.0 \mu$, glabris vel delicate asperatis, in catenis laxae parallelibus usque 200μ longis.

In culturis e corio mucoso, Philadelphia, Pa.

Colonies on Czapek's solution agar extremely slow-growing (FIG. 11A), 2 to 5 mm. in twelve to fourteen days at room temperature, thin or consisting of a fairly tough mycelial felt, surface appearing velvety or slightly granular, medium sporing in yellow-green shades near Andover green (Ridgway, Pl. XLVII); exudate lacking; odor suggesting sea-weed; reverse uncolored; conidiophores arising from the mycelial felt, up to 200μ by 2.0 to 2.5μ , with walls smooth or nearly so; penicilli typically biverticillate and symmetrical (FIG. 11C), regularly consisting of a terminal verticil of 5 to 7 or 8 metulae measuring about 9 to 11μ by 2.0 to 2.5μ , slightly enlarged upward; sterigmata usually in compact clusters of 6 to 8, mostly 8 to 10μ by 1.8 to 2.2μ ; conidia at first elliptical, becoming subglobose or broadly elliptical when mature, with walls thin, smooth or delicately roughened, mostly 2.0 to 2.5μ by 1.5 to 2.0μ , borne in tangled chains up to 75 or 100μ in length.

Colonies on steep agar essentially as on Czapek but usually lighter sporing, conidial structures sparsely produced, often smaller than on Czapek.

Colonies on malt extract agar spreading broadly, up to 5.0 to 5.5 cm. in twelve to fourteen days, velvety, plane, with vegetative mycelium largely submerged, bearing abundant conidial structures in a dense stand (FIG. 11B), consistently narrowly zonate, heavily sporing throughout in dull gray shades near grayish olive (R., Pl. XLVI); showing abundant short, encrusted and pigmented hyphae intermixed with conidial structures; exudate lacking; odor not pronounced, slightly musty; reverse uncolored; conidiophores up to 300μ in length; penicilli as described on Czapek but generally showing metulae and sterigmata slightly longer; conidia in loosely parallel chains up to 200μ in length.

Colonies on Czapek's solution agar containing ammonium sulfate (2.33 g./liter) as the nitrogen source approximating those on malt in rate of growth, general texture, and in the abundance of conidial structures produced; conidial areas near Andover green (R., Pl.

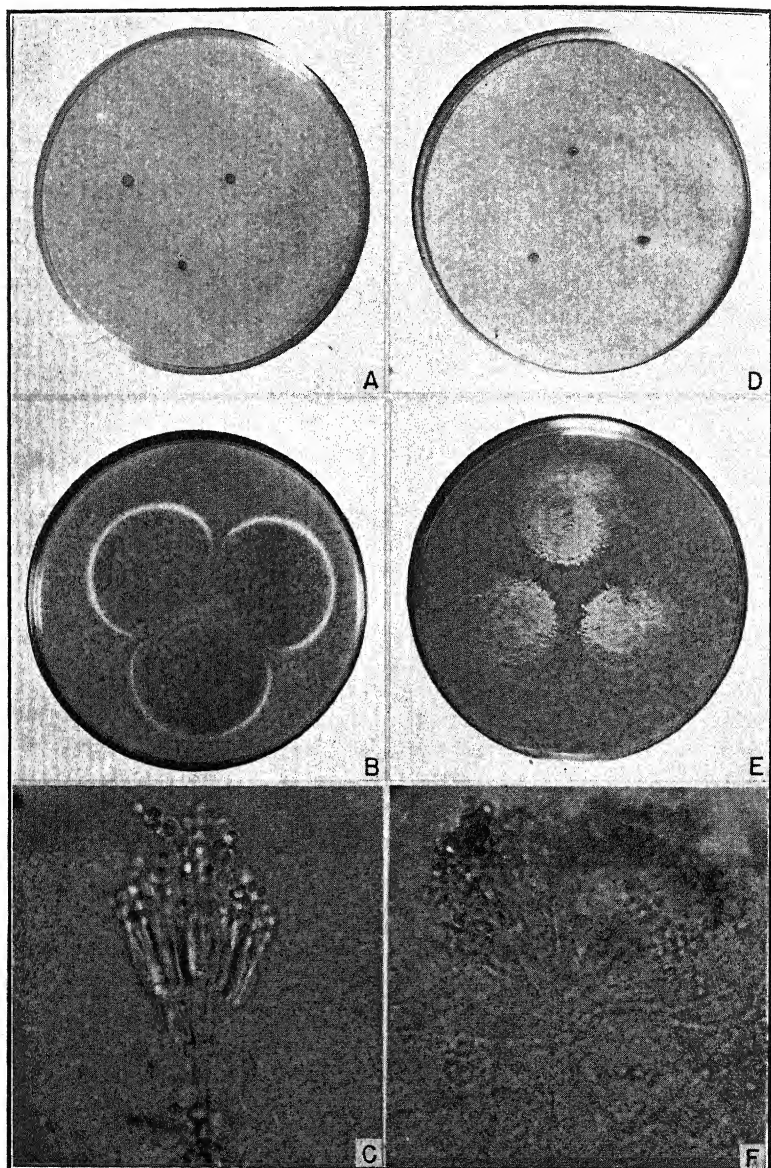


FIG. 11. *Penicillium diversum* and var. *aureum*.

XLVII), showing a limited development of sterile, yellow-pigmented aerial hyphae in submarginal areas; exudate lacking; odor indistinct; reverse uncolored; penicilli as described on malt agar.

Species description centered upon NRRL 2121 as **type**, isolated from moldy leather and submitted to us for identification by Dr. T. C. Cordon, Eastern Regional Research Laboratory; and NRRL 2122, isolated from soil collected in Sweden and contributed by Professor Edy Velander, Stockholm. The species is represented by numerous additional strains isolated from deteriorating military equipment, dried egg powder, and soils.

This species is placed in the series with *Penicillium tardum* Thom (1930) primarily because of its very restricted growth upon standard Czapek and steep agars that are neutral in reaction and contain sodium nitrate as the principal nitrogen source. The fact that the species grows luxuriantly upon malt extract agar and upon Czapek's solution agar containing ammonium sulfate clearly demonstrates the presence of some nutrient deficiency. The species is favored by a reaction of pH 4.0 to 6.0, but grows very sparsely and fails to sporulate at pH 7.0. Recognition of the species as new is based not upon its inability to utilize nitrate nitrogen, but upon its failure to duplicate any recognized species even upon favorable media, such as malt extract agar, or Czapek agar containing ammonium nitrogen, where it grows luxuriantly. The species is clearly different from *Penicillium tardum* Thom: penicilli are more consistent in pattern; conidia are smaller, less definitely elliptical, thin-walled, and smooth; and colonies upon favorable media are spreading, plane, heavy sporing, velvety, and narrowly zonate.

The binomial, *Penicillium diversum* (from the Latin word *diversus*), is based upon the markedly different growth of this species upon different substrata.

***Penicillium diversum* var. *aureum* var. nov.**

Colonies on Czapek's solution and steep agars duplicating those of the species in color, texture, and rate of growth (FIG. 11D); producing a fairly dense stand of conidial structures which differ

from the species in showing metulae and sterigmata more numerous in the verticil and usually somewhat shorter.

Colonies on malt agar duplicating those of the species in rate of growth but differing markedly in color and texture (FIG. 11E), approximating olive-yellow to olive-ocher (Ridgway, Pl. XXX), appearing somewhat granular or tufted, especially in marginal areas, and consisting of a comparatively thin, closely interwoven network of yellow encrusted, much-branched, sterile hyphae enmeshing and largely obscuring numerous conidial structures; penicilli biverticillate and symmetrical, short and very compact (FIG. 11F), bearing metulae in large crowded clusters of 12 to 15 or more, individually measuring about 7 to 8 μ by 1.5 to 1.8 μ ; sterigmata in verticils of 6 to 8, about 7.5 to 9.0 μ by 1.5 μ , lanceolate with characteristically tapered tips; metulae and sterigmata usually yellowish green in color; conidia elliptical to subglobose, mostly 2.4 to 2.8 μ by 2.0 to 2.5 μ with walls comparatively thin, smooth or nearly so, in yellow-green shades.

The varietal name is based upon a characteristic yellow coloration upon certain media that are unusually favorable for growth, including malt extract and Sabouraud's agars. The yellow variety differs from the typical variety in two outstanding characteristics: (1) The production of greatly increased amounts of yellow encrusted mycelium, and (2) the production of larger penicilli consisting of substantially greater numbers of metulae and sterigmata.

Growth is more luxuriant when cultivated upon media containing ammonium rather than nitrate nitrogen and it is greater on acid than neutral substrata. The variety does not grow as well upon Czapek agar containing ammonium sulfate as upon malt agar. It does, however, seem to possess the same basic nutritional deficiencies as the species.

The variety is represented by NRRL 1074 as **type**, received in 1934 from Ross W. Davidson, Division of Forest Pathology, Bureau of Plant Industry. The strain was initially diagnosed as *Penicillium tardum* Thom upon the basis of its symmetrically biverticillate penicilli and its restricted growth upon Czapek's agar. The subsequent discovery and recognition of *P. diversum* showed the probable relationship of the variety to be with the latter species rather than with *P. tardum* Thom.

SUMMARY

Eleven species and one variety of *Penicillium* are described as new. The new forms include:

1. Five ascosporic species:

- a. *Penicillium parvum* and *P. levitum* produce monoverticillate penicilli and perithecia that are at first pseudoparenchymatous throughout. They are assignable to the ascosporic series typified by *P. javanicum* v. Beyma.
- b. *Penicillium rotundum* and *P. helicum* unmistakably represent members of the *P. luteum* series and produce soft, loose-textured perithecia. Conidial structures, although often fractional, are typically biverticillate and symmetrical with lanceolate sterigmata.
- c. *Penicillium striatum* produces perithecia of the type characteristic of the *P. luteum* series, but fails to develop either symmetrical penicilli or lanceolate sterigmata. Its relationship is somewhat doubtful.

2. One sclerotigenic species, *Penicillium lapidosum*, with penicilli typically monoverticillate. It is closely related to *P. thomii* Maire.

3. Five non-ascosporic species and one variety:

- a. *Penicillium capsulatum* with monoverticillate penicilli borne on short branches from ascending hyphae, hence assignable with other ramigenous species.
- b. *Penicillium lavendulum* produces asymmetric penicilli with cellular elements conspicuously echinulate; it is assignable to the Asymmetrica-Funiculosa near *P. pallidum* Smith.
- c. *Penicillium piceum*, *P. aculeatum*, *P. diversum*, and *P. diversum* var. *aureum* represent members of the Biverticillata-Symmetrica section. These are assignable to series typified by the well-recognized species, *P. funiculosum* Thom, *P. purpurogenum* Stoll, and *P. tardum* Thom, respectively.

The writers are indebted to Dr. Charles Thom for his counsel and advice relative to the validity and relationships of these species; and to Miss Edith K. Cash for preparing the Latin diagnoses.

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EXPLANATION OF FIGURES

FIG. 1. *Penicillium parvum*; NRRL 2095. *A* and *B*, Two-week-old colonies on Czapek's solution and hay infusion agars, respectively. *C*, Low-power view of perithecia on corn meal agar, $\times 60$. *D*, Ascospores, $\times 1500$; note small dimensions and prominent equatorial ridges and furrows.

FIG. 2. *Penicillium levitum*; NRRL 705. *A* and *B*, Two-week-old colonies on Czapek's solution and malt agar, respectively. *C*, Detail of penicilli, which are typically monoverticillate and often fractional, $\times 800$. *D*, Broken perithecia showing ascogenous masses extruded by pressure of the coverglass, $\times 85$. *E*, Asci in various stages of development. *F*, Ascospores, $\times 1500$; note comparatively heavy, smooth walls.

FIG. 3. *Penicillium helicum*; NRRL 2106. *A*, Ten-day-old colonies on malt extract agar. *B*, Initial stage in perithecium development; note the thick hypha (ascogonium?) with terminal area coiled in characteristic fashion, and (*b*) the smaller hypha (antheridium?) wound around the basal portion of it. *C*, Perithecia as developed on malt extract agar, $\times 30$. *D*, Ascospores, $\times 1500$; the delicately spinulose character of the spore walls is not shown.

FIG. 4. *Penicillium rotundum*; NRRL 2107. *A*, Ten-day-old colonies on malt extract agar. *B*, Initial stage in perithecium development; unlike *P. helicum*, this species shows no regularity in pattern and fails to develop consistent structures identifiable as possibly representing ascogonia and antheridia. *C*, Perithecia as developed on malt agar, $\times 30$. *D*, Ascospores, $\times 1500$; note characteristic globose form and conspicuously roughened walls.

FIG. 5. *Penicillium striatum*; NRRL 717. *A* and *B*, Two-week-old colonies on Czapek's solution and malt extract agars, respectively. *C*, Conidial stage: *C*₁, penicilli as seen under low power, $\times 100$; *C*₂, detail of penicilli showing cellular patterns and arrangements, $\times 800$; *C*₃, mature conidia, $\times 800$. *D*, Perithecium compressed by a coverglass, showing extrusion of asci through the surrounding loose hyphal envelope, $\times 450$. *E*, Asci, showing the manner in which these are borne singly on short branches from the fertile hyphae, $\times 900$. *F*, Ascospores, showing the prominent longitudinal frills which characterize this species, $\times 900$.

FIG. 6. *Penicillium lapidosum*; NRRL 718. *A* and *B*, Two-week-old colonies on Czapek and malt agars, respectively. *C*, Sclerotia as seen on corn meal agar, $\times 65$. *D*, Detail of penicillus, $\times 900$.

FIG. 7. *Penicillium capsulatum*; NRRL 2056. *A* and *B*, Two-week-old colonies on Czapek and malt agars, respectively. *C*, Low-power view showing origin of conidial structures from ascending hyphae, $\times 100$. *D*, Detail of penicilli, $\times 900$.

FIG. 8. *Penicillium lavendulum*; NRRL 2146. *A* and *B*, Two-week-old colonies on Czapek and malt agars, respectively. *C*, Low-power view of penicilli on malt agar, $\times 75$. *D*, Detail of penicilli, $\times 900$.

FIG. 9. *Penicillium piceum*; NRRL 1071. *A* and *B*, Ten-day-old colonies on Czapek and malt agars, respectively. *C*, Conidial heads as seen under low-power presenting the spruce-tree-like pattern that is characteristic of this species, and from which the name is taken, $\times 115$. *D* and *E*, Penicilli under successively increased magnification, showing origin and details of structure, $\times 190$ and $\times 900$, respectively.

FIG. 10. *Penicillium aculeatum*; NRRL 2130. *A* and *B*, Ten-day-old colonies on Czapek and malt agars, respectively. *C*, Detail of penicilli, showing rough conidiophores and globose, conspicuously roughened conidia, $\times 1000$.

FIG. 11. *A-C*, *Penicillium diversum*; NRRL 2121. *A* and *B*, Ten-day-old colonies on Czapek and malt agars, respectively. *C*, Detail of penicillus, $\times 900$. *D-F*, *Penicillium diversum* var. *aureum*; NRRL 1074. *D* and *E*, Ten-day-old colonies on Czapek and malt agars, respectively. *F*, Detail of penicillus, showing numerous metulae, $\times 700$.

STUDIES IN THE GASTEROMYCETES: XVI. THE GEASTRACEAE OF THE SOUTH- WESTERN UNITED STATES

W. H. LONG AND DAVID J. STOFFER

(WITH 32 FIGURES)

This paper is a report on plants belonging to the family Geastraceae collected over a period of several years, mainly by us, in the states of Arizona, New Mexico and Texas.

KEY TO GENERA

1. Endoperidium with a prominent sterile base.....*Terrostella*
1. Endoperidium without a sterile base.....2
2. Endoperidium normally with one mouth, persistent.....3
2. Endoperidium normally with several mouths.....*Myriostoma*
2. Endoperidium caducous, with a persistent subligeous columella.
Trichaster
3. Columella present, threads of capillitium simple.....*Geaster**
3. Columella none, the threads of capillitium much branched.....*Astraeus*

The genus *Trichaster* has not yet been found in this region; hence only the four remaining genera are discussed in this paper.

All of the descriptions and photographs used in this article were made from dried herbarium material, since we rarely find any fresh "green" plants in our hot, semi-arid region; furthermore the "green" plants are not satisfactory for taxonomic purposes since they normally do not show many of the salient characters found in the usual herbarium material which we have had to use in comparing and differentiating the various species.

GEASTER Mich. Nova Plantarum Genera, p. 220. 1729

Sporophore hypogeous or epigeous, globose to acuminate; *exoperidium* of three well defined layers, an outer mycelial layer, a mid-

*The late Dr. Long did not follow the *International Rules* and consequently did not recognize Persoon's Syn. Meth. Fungorum as the starting point for the nomenclature of this genus. The correct spelling and citation for the genus in accordance with the Rules is *Geastrum* Pers., Syn. Method. Fung., p. 131. 1801.—Editor.

dle fibrillose layer and an inner collenchyma one, at first closely investing the endoperidium, at maturity splitting in a stellate manner. *Endoperidium* sessile or pedicellate, usually dehiscing by a single apical mouth. *Columella* present or lacking in the mature stage. *Capillitium* threads usually simple, long and tapering. *Spores* globose to subglobose. *Epispore* smooth or verrucose (Adapted from Kambly and Lee).

This paper reports three species of *Geaster* not previously known in the United States; namely *G. Hariotii*, *G. elegans* and *G. Hieronymi*, also two new species, *G. pluriosteus* and *G. xylogenus*.

KEY TO SPECIES OF GEASTER DISCUSSED IN THIS PAPER

1. Mouth sulcate.....2
1. Mouth fibrillose.....5
1. Mouth naked.....7
2. Plants strongly hygroscopic, spore sac sessile....1. *Geaster Drummondii*
2. Plants not hygroscopic.....3
2. Plants subhygroscopic.....4
3. Mouth long beaked, pedicel slender.....2. *G. pectinatus*
3. Mouth long beaked, base of spore sac with a collar-like ring.
3. *G. Bryantii*
3. Mouth short beaked, pedicel short, thick.....4. *G. Schmidelii*
3. Plants medium size, explanate, spore sac sessile.....5. *G. Hariotii*
3. Plants small, spore sac sessile, vaulted.....6. *G. elegans*
3. Spore sac with several mouths—smooth.....7. *G. pluriosteus*
3. Spore sac subsessile, densely furfuraceous, rays truncate..8. *G. xerophilus*
4. Spore sac smooth, mouth flattened conical, sulcate-striate..10. *G. Smithii*
4. Spore sac rough, asperate.....9. *G. campestris*
4. Plants subsessile, subsaccate, spore sac smooth.....13. *G. arenarius*
5. Plants strongly hygroscopic, spore sac sessile.....11. *G. mammosus*
5. Plants not hygroscopic.....6
6. Plants medium size, epixylous, epigeous.....12. *G. xylogenus*
6. Plants small, sessile, saccate, tomentose.....14. *G. tomentosus*
6. Plants pedicellate, smooth, 8–12 rays.....15. *G. minimus*
6. Plants small, 4–5 rays, fornicate.....16. *G. coronatus*
6. Plants typically large, spore sac strongly asperate.....17. *G. Hieronymi*
6. Plants large, spore sac smooth with apophysis.....18. *G. limbatus*
6. Plants small to medium, spore sac sessile, saccate.
19. *G. saccatus* form *minor*
6. Plants large to medium, subsaccate, rays acuminate.....20. *G. triplex*
6. Plants medium size to small subsaccate, rays acuminate.
21. *G. saccatus* form *major*
7. Plants strongly hygroscopic, spore sac sessile.....25. *G. floriformis*
7. Plants strongly hygroscopic, spore sac sessile, mouth a torn aperture.
Astracrus hygrometricus
7. Plants not hygroscopic.....8

8. Rays 4-5, plant pedicellate, fornicate.....22. *G. fornicatus*
 8. Rays 5-6, plants large, explanate with hypophysis.....23. *G. rufescens*
 8. Plants small to medium, subsaccate, sessile.....24. *G. fimbriatus*

1. *GEASTER DRUMMONDII* Berk. Lond. Jour. Bot. 4: 63. 1845
 (FIG. 1)

Sporophore hypogeous with a universal mycelium, becoming superficial and expanded at maturity, then 2-3 cm. in diameter. *Exoperidium* revolute when wet, split nearly to middle. Rays 8-10, subequal, acute, rigid, strongly hygroscopic, usually involute over the spore sac, covering it more or less entirely, with tips slightly revolute when wet. *Fleshy layer* adnate, continuous, not rimose or splitting, pecan brown to Vandyke brown. *Exterior* at first covered with a thin layer of mycelium and dirt, usually peeling off and leaving the outer surface of the fibrillose layer clean and free of dirt, usually dingy white. *Base* plane to convex when rays are expanded, not umbilicate. *Endoperidium* sessile, membranous, 8-15 mm. across, wood brown to light drab to benzo brown, minutely furfuraceous, soft to slightly asperate. *Mouth* usually concolorous with endoperidium, sulcate, acute, conic *sulci* of uneven length, usually branched at base, often wrinkled, 16-20 for long ones. *Gleba* seal brown. *Columella* subglobose to inevident.

Habitat: Solitary to usually gregarious in small groups in partial shade of desert or other vegetation.

Distribution: ARIZONA, near Prescott, 5500 ft., 1-2-34, *W. H. Long*, 2 plants, 7906; 9-15-33, 1 plant, 7760; Eagle Ranger District, Crook Nat. For., *D. J. Stouffer*, 3-24-47, 4 plants, 11455 and 3-28-47, 19 plants, 10104. Prescott, 2-16-34, *W. H. Long & V. O. Sandberg*, 5 plants, 7615. NEW MEXICO, Lincoln County, Jicarilla, 2-29-41, *D. J. Stouffer*, 11 plants, 9320; 5-21-41, 23 plants, 9434; 7-18-41, 10 plants, 9542; Cougar Mt., August, '41, 25 plants, 9513; Cougar Tank, 5-28-41, 22 plants, 9429; Corona area, 9-6-41, *W. H. Long*, 12 plants, 9597; 4-20-42, 24 plants, 10220; 9-6-41, 18 plants, 9563; Ranger Tank, 9-5-41, *W. H. Long & D. J. Stouffer*, 20 plants, 9529; 9-15-41, 2 plants, 9663; 5-19-40, *D. J. Stouffer*, 15 plants, 9430; in sandhills near Gran Quivera Road, west of Corona, Sept. '41, 5 plants, 9844; Corona area, 4-17-42, *W. H. Long*, 6 plants, 10072; 8 mi. south of Oscura, 2-17-42, *D. J. Stouffer*, 3 plants, 10020; 4-18-42, *W. H. Long & D. J. Stouffer*, 16 plants, 10244; Jornada Exp. Range, 9-8-41, *W. H. Long & D. J. Stouffer*, 4 plants, 9713; 10-2-39, *W. H. Long*, 6 plants, 8399; Corona area, 9-4-41, *W. H. Long & D. J. Stouffer*, 13 plants, 9508; near Magdalena, Tres Montosas Mts., 10-11-34, *A. E. Frazier*, 6 plants, 8895; Jornada Exp. Range, 9-7-41, *W. H. Long & D. J. Stouffer*, 3 plants, 9594; Corona area, 4-15-42, *W. H. Long*, 4 plants, 11444. TEXAS, Denton, 10-22-01, *W. H. Long*, 1 plant, 1081; 12-20-02 (Long no. 7482), 3 plants in Lloyd Myc. Coll. no. 22723.

2. *GEASTER PECTINATUS* Pers. Syn. Meth. Fung., p. 132. 1801
(FIG. 2)

Sporophore hypogeous in leaf debris, with a universal mycelium, superficial at maturity, then revolute to vaulted. *Exoperidium* revolute, strongly vaulted, split about to the middle, 1-4 cm. across when expanded. *Rays* 10-12, pliable, not hygroscopic, very unequal, narrow, acute. *Fleshy layer* usually adnate, not rimose, wood brown to bister. *Exterior* covered with leaf debris. *Base* concave to often vaulted, no umbilical scar. *Endoperidium* pedicellate, pedicels slender, 2-3 mm. long, not striate, subglobose, 5-12 mm. wide by 8-12 mm. tall, with or without apophysis, lead or plum colored (dark plumbago slate) to Prout's brown, often with a white pruinose covering which easily rubs off, smooth. *Peristome* strongly sulcate, beaked or slender conical with 16-20 sulci, walnut brown, seated in a more or less definite depressed area. *Gleba* seal brown to blackish brown. *Columella* inevident. *Capillitium* light brown, unbranched, hyphae of capillitium 3-7 μ thick. *Spores* globose, brown, 4-6 μ in diameter. *Epispore* verrucose.

Habitat: In leaf debris of pines and junipers.

Distribution: ARIZONA, Eagle District, Crook Nat. For., 3-24-47, D. J. Stouffer, 2 plants, 11443. NEW MEXICO, Corona area, Sept. '40, D. J. Stouffer, 2 plants, 9131; 4-1-41, 5 plants, 9281; 1-14-42, 1 plant, 9989; 9-6-41, W. H. Long, 1 plant, 9813; W. H. Long & D. J. Stouffer, 4-20-40, 4 plants, 9680; 9-17-41, 1 plant, 9781; 25 miles west of Corona, 12-15-41, D. J. Stouffer, 2 plants, 9964; Lincoln County, Jicarilla, 9963; 9-16-41, W. H. Long & D. J. Stouffer, 2 plants, 9793.

3. *GEASTER BRYANTII* Berk. Outl. Brit. Fungi, p. 300. 1860
(FIG. 3)

Sporophore hypogeous in leaf debris with a universal mycelium, superficial at maturity, then becoming explanate to revolute. *Exoperidium* revolute to often involute, split to about the middle, 1.5-3 cm. across when expanded. *Rays* 8-12, pliable, not hydroscopic, very unequal, some involute at base of spore sac. *Fleshy layer* usually adnate, not rimose, wood brown to bister. *Exterior* covered with a thin layer of leaf debris, held by the mycelial layer. *Base* concave, often vaulted, no umbilical scar. *Endoperidium* pedicellate, pedicels slender, 1-3 mm. long, not striate, subglobose, 5-12 mm. wide by 5-10 mm. tall, with a well defined collar or ring around the base of the spore sac just above the top of the pedicel, lead to plum color (dark plumbeous slate) to light brown, usually with a pronounced white pruinose covering which easily rubs off.

Peristome strongly sulcate, beaked or slender conical, 12-16 sulci, 4-5 mm. tall, walnut brown, seated in a more or less depressed area. *Gleba* seal brown. *Columella* inevident. *Capillitium* brown, unbranched. *Spores* globose, 4-5.5 μ in diameter. *Epispore* distinctly verrucose.

Habitat: In leaf debris under junipers and mesquite trees.

Distribution: ARIZONA, 7 miles from Nogales, 2-19-34, W. H. Long & V. O. Sandberg, 9 plants, 7624. NEW MEXICO, Corona area, 9-14-41, W. H. Long & D. J. Stoutter, 9 plants, 9665. TEXAS, Denton, 12-23-07, W. H. Long, 2 plants, 2060; 1901, ex Herb. C. L. Shear, 2 plants now in Lloyd Myc. Coll. no. 31143; 10-22-01 (Long no. 1081), 9 plants ex Herb. C. L. Shear; 1-12-03 (Long no. 1807), 24 plants in Lloyd Myc. Coll. no. 51909.

4. GEASTER SCHMIDELII Vitt. Mon. Lyc. p. 12. 1842 (FIG. 4)

Sporophore hypogeous in leaf debris with a universal mycelium, superficial and expanded at maturity. *Exoperidium* revolute, vaulted, split to about the middle, 1-2.5 cm. across when expanded. *Rays* 6-10, pliable, revolute, rarely involute under the spore sac, not hygroscopic, very unequal, many narrow, acute. *Fleshy layer* usually adnate, not rimose, wood brown when fresh becoming army brown to dingy white in age. *Exterior* covered with a dense layer of leaf debris. *Base* concave, often vaulted, no scar. *Endoperidium* subsessile to short pedicellate, pedicels stout, 1 mm. long or less, usually white, subglobose, 5-10 mm. wide by 6-12 mm. tall, apophysis none, wood brown to tilleul buff, rarely plumbeous black. *Peristome* strongly sulcate, short beaked or only slender conical, with 12-18 sulci, usually darker than exoperidium, seated in a more or less depressed area. *Gleba* seal brown. *Columella* inevident. *Capillitium* light brown, unbranched. *Spores* globose, medium dark, 4-5 μ in diameter. *Epispore* verruculose.

Habitat: In leaf debris under junipers.

Distribution: ARIZONA, Sabino Canyon area, near Tucson, 9-28-39, W. H. Long, 1 plant, 8774. NEW MEXICO, Corona area, D. J. Stoutter, 4-4-40, 4 plants, 9266; 5-19-41, 4 plants, 9446; 6-16-41, 13 plants, 9435; W. H. Long & D. J. Stoutter, 4-20-40, 8 plants, 8706; 9-4-41, 1 plant, 10012; 9-5-41, 1 plant, 9807; 4-17-42, 20 plants, 10077; Jornada Exp. Range, Sept. '41, 2 plants, 9591; Cougar Tank, 5-28-40, D. J. Stoutter, 4 plants, 9445; 20 mi. N.W. of Corona, 7-23-41, 3 plants, 9552. TEXAS, Denton, W. H. Long, 1-12-13 (Long no. 1795), 4 plants, Lloyd Myc. Coll. no. 51912; 7-17-1900, 6 plants, 11199; Shoal Creek, 7-17-1900, 2 plants, 11180; Waller Creek, 3-2-03, W. H. Long & A. M. Ferguson, 3 plants, 11197 (Long no. 1816); 1900-1901, 10 plants, Lloyd's no. 31571.

This species as it grows in this area is smaller than either *G. pectinatus* or *G. Bryantii*.

5. *GEASTER HARIOTII*, Lloyd Myc. Writ. 2: 311. 1907 (FIG. 5)

Sporophore hypogeous, becoming superficial and expanded at maturity, then 2–3 cm. in diameter. *Exoperidium* explanate, split about two thirds of the way to middle. *Rays* 6–8 explanate, pliable, not hygroscopic, unequal, broad to narrow, acute. *Fleshy layer* adnate, not rimose or splitting off, snuff brown to Rood's brown. *Exterior* covered with a thin layer of soil (which is peeling off on one plant), cartridge buff on the naked under surface. *Base* plane to convex to slightly concave, no umbilical scar. *Exoperidium* sessile, subglobose, 6–12 mm. wide, no signs of pits, smooth, sayal brown to avellaneous. *Mouth* sulcate, slightly protruding, circular, acute. *Peristome* small with uneven sulci 20–30, sayal brown, darker than endoperidium. *Gleba* ferruginous. *Capillitium* hyaline to slightly tinted, walls very thin, 4.4–5.5 μ , outer wall rough, walls chestnut color. *Spores* globose, dark brown, 4–5 μ diameter. *Epispore* verrucose.

Habitat: In leaf debris under *Juniperus sabinooides*.

Distribution: TEXAS, Austin, 11–23–03, A. M. Ferguson, 3 plants, 11196.

This is apparently a pale form of *G. Hariotii* Lloyd, and corresponds very closely to one of the plants in Lloyd's no. 52536, which has 2 plants, one dark and typical and the other a much lighter color, collected by Gaudichaud in Rio de Janeiro, Brazil.

6. *GEASTER ELEGANS* Vitt. Mon. Lyc. p. 159, 1843 (FIG. 6)

Sporophore hypogeous, becoming superficial and expanded at maturity, 1–2 cm. across. *Exoperidium* revolute, vaulted, split to about the middle. *Rays* 6–11, subequal, acute, revolute, not hygroscopic, pliable. *Fleshy layer* adnate, not rimose, army brown. *Exterior* covered with a thin layer of dirt. *Base* concave, vaulted, no umbilical scar. *Endoperidium* sessile, sometimes subsaccate, 0.5–1 cm. across, smooth, with a soft furfuraceous reddish mat, brownish to dingy white after wintering, subglobose, seated in a slight depression. *Peristome* sulcate, protruding, dark brown, 6–8 equal sulci. *Gleba* cinnamon. *Columella* inevident. *Capillitium* light brown, thinner than the spores. *Spores* globose, 5–7 μ

diameter, uniguttulate. *Epispore* strongly verrucose, some appearing semireticulate.

Habitat: Gregarious in juniper and pine debris.

Distribution: ARIZONA, Flagstaff, in ponderosa pine duff, 2-6-34, W. H. Long, 3 plants, 10030. NEW MEXICO, Corona area, 2-14-41, D. J. Stouffer, 10 plants, 9254; near Ranger Tank, 5-12-41, 3 old plants, 9325.

We compared these plants with *Geaster elegans* no. 51906 Lloyd Myc. Coll. from Alliers, France, collected by Rev. H. Bourdot, and found them to be the same. See Lloyd's plate 99, figures 5a and 5b.

7. *Geaster pluriosteus* sp. nov. (FIG. 7)

Sporophoro initio hypogaeo dein epigaeo. *Exoperidio* usque ad medium in 5-6 laciniis inaequalibus, acutis fisso, explanato vel recurvato, flaccido. *Endoperidio* 6-15 mm. lato, 5-8 mm. alto, globoso vel depresso-globoso, sessili, glabro. *Peristomio* plicati-sulcato, subconico, 6-15 mm. lato, 5-8 mm. alto. *Oribus* singulis usque pluribus, circularibus. *Sporis* globosis, verrucosis, 4-5 μ diam.

Sporophore hypogeous with a universal mycelium becoming superficial at maturity, then more or less explanate, 1.5-2 cm. across when fully expanded. *Exoperidium* explanate to slightly revolute, split one half to two thirds of the way to middle. *Rays* 5-6, pliable, not hygroscopic, very unequal, acute. *Fleshy layer* adnate, very thin when dry, not rimose or cracking, pecan brown. *Exterior* covered with a thin layer of sand. *Base* slightly concave, no umbilical scar. *Endoperidium* sessile, subglobose to depressed-globose, 6-15 mm. wide by 5-8 mm. tall, smooth, light drab when unweathered. *Mouth*, one to several, circular to elliptic, sulcate, short, acute, with 5-8 weak sulci, equal, extending to apex of peristome, drab to mouse gray, not in a depressed area, darker than balance of endoperidium. *Peristome* rather indeterminate, covered with close whitish granules which are not asperate and rub off rather easily in handling. *Gleba* cinnamon. *Columella* sepia. *Capillitium* colored, walls thin, about 5 μ thick. *Spores* globose, 5.5-7 μ diameter. *Epispore* coarsely verrucose.

Habitat: Gregarious in leaf debris of *Juniperus monosperma* but no leaf debris attached, only a thin layer of sand.

Distribution: NEW MEXICO, 5-6 miles N.E. of Corona, 9-17-41, W. H. Long & D. J. Stouffer, 6 plants, no. 9772; Lincoln County, Jicarilla, 9-15-41, 1 plant, no. 9258.

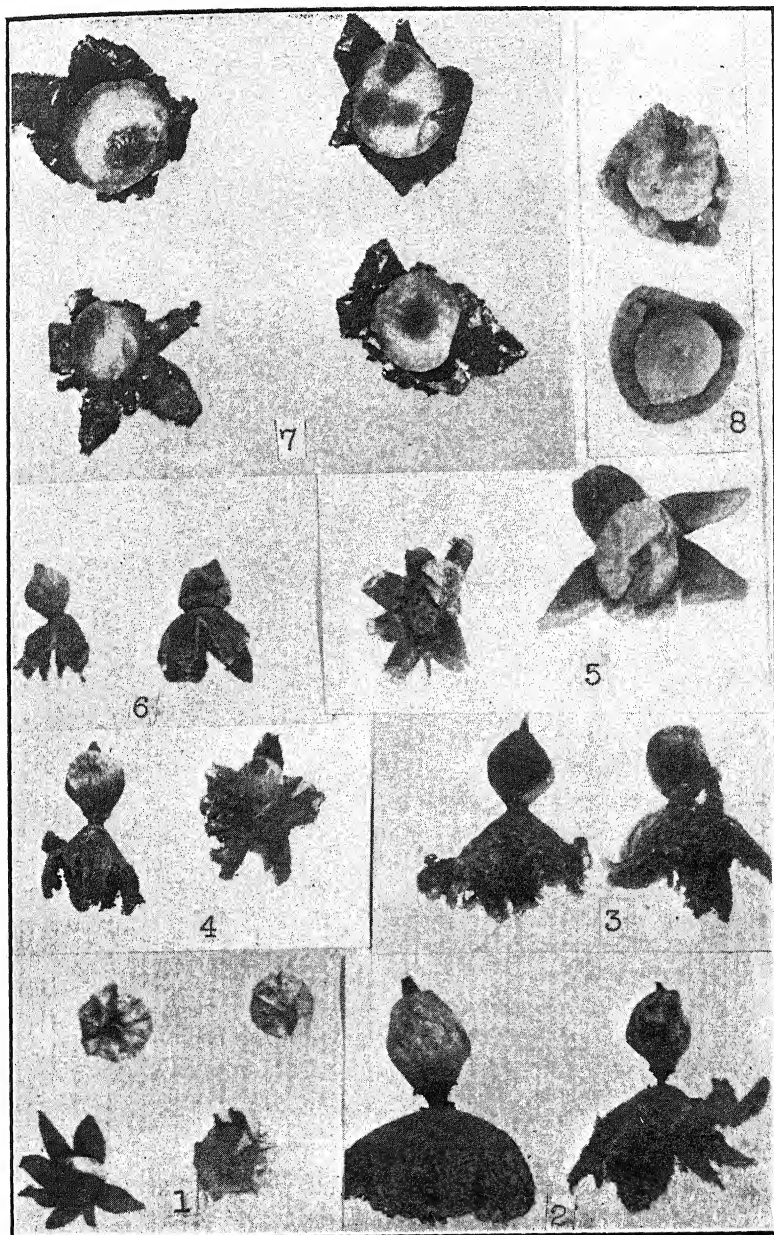
This is the only species of *Geaster* that we have ever seen with more than one mouth, although Cunningham (1944) reports a species—*G. ellipticus*, from Australia—that sometimes has two in the same fruiting body, but our plant apparently is a different species.

8. *GEASTER XEROPHILUS* Long, *Mycologia* 34: 13–16. 1942
(FIG. 8)

Sporophore hypogeous, small, the button subglobose to strongly depressed-globose, or rarely concave on top, 1–2 cm. across, having a strong basal mycelial cord, becoming superficial and expanded at maturity, then 1–4 cm. in diameter, usual size about 2 cm. *Exoperidium* saccate to explanate, split to about the middle or rarely to center in old weathered plants. *Rays* 7–12, pliable not hygroscopic, unequal, blunt to truncate, tardily expanded or with tips involute around the spore sac. *Fleshy layer* cream buff to cinnamon, adnate, continuous, rarely rimose. *Exterior* covered with sand held by the persistent, thin, strongly adnate mycelial layer which even in very old weathered plants is still retained. *Base* concave with a prominent umbilical scar. *Endoperidium* sessile to usually short-pedicellate, subglobose to strongly depressed-globose, often flattened on top (watch-shaped), 1–2 cm. across, light buff to drab gray when unweathered, becoming pallid mouse gray to pale cartridge buff (whitish) with age, densely and minutely furfuraceous (but not asperate), lower one third often enclosed by the saccate base of the exoperidium. *Peristome* small, usually circular, sometimes elliptic, acute, sulcate with 18–30 sulci of unequal thickness and length, some not extending to apex of peristome, not seated in a depressed area, concolorous or rarely darker, in age often becoming enlarged into a gaping mouth. *Gleba* mummy brown when not weathered but becoming snuff brown with age. *Columella* prominent, cylindrical, expanding at top into a persistent globose mass of hyphae. *Capillitium* simple, snuff brown, 4–4.5 μ thick. *Spores* globose, uniguttulate, 4–5 μ diameter, semiopaque in water. *Epispore* chestnut brown, coarsely verrucose.

Habitat: Solitary or in small groups, in open sandy area rarely in partial shade of desert plants; in hot dry regions.

Distribution: This is a continuation of the distribution given in the previous paper by Long (1942) but not included in it. NEW MEXICO, Jornada Exp. Range, 9–7–41, W. H. Long & D. J. Stouffer, 8 plants, 9593; 9–8–41,



FIGS. 1-8, $\times 1$. *Geaster*; 1, *G. Drummondii*, 4 plants; 2, *G. Bryantii*, 2 plants; 3, *G. pectinatus*, 2 plants; 4, *G. Schmideli*, 2 plants; 5, *G. Hariotii*, 2 plants; 6, *G. elegans*, 2 plants; 7, *G. pluriosteus*, 4 plants; 8, *G. xerophilus*, 2 plants.

10 plants, 9713; Oscuro, 4-18-42, 8 plants, 10243; White Sands Nat. Monument, 8-22-42, *W. H. Long*, 1 plant, 10207; near Belen, 9-24-41, 6 plants, 9725; 12-6-41, *W. H. Long & D. J. Stouffer*, 9 plants, 9922; 4-15-42, 2 plants, 10230; Oscuro, 2-17-42, *D. J. Stouffer*, 4 plants, 10019; 4-18-42, *W. H. Long & D. J. Stouffer*, 5 plants, 10090; 34 miles east of Roswell, in Oak Shinnery, 4-19-42, 10 plants, 10089; 10 miles west of Deming, 6 plants, 9830; 9-13-41, 5 plants, 9657; 4 plants, 9642; 4-24-42, *W. H. Long*, 10 plants, 10066; Bernalillo area, 8-24-41, 9 plants, 9470; 9-8-41, *W. H. Long & D. J. Stouffer*, 6 plants, 9508; Albuquerque area, 1-27-42, *W. H. Long*; 2 plants, 9988; 8-23-41, 2 plants, 9466; 2 plants, 9999; 10-16-41, 1 plant, 9821; 8-30-41, 19 plants, 9485; 9-1-41, 9 plants, 9488; 11-27-41, 11 plants, 9912; 1-19-42, 20 plants, 9980; 4-4-42, 2 plants, 10051; 8-29-41, 10 miles south of Albuquerque, 4 plants, 9478; Parida Canyon near Willard, 11-29-41, *D. J. Stouffer*, 1 plant, 9945; 25 miles N.W. of Corona, 11-20-41, 9 plants, 9930; 12-15-41, 2 plants, 9962; Gran Quivera road, west of Corona, Sept. '41, 1 plant, 9842; in sand-hill-juniper area 25 miles from Corona, 9-17-41, *W. H. Long & D. J. Stouffer*, 4 plants, 9741; Corona area, 9-4-41, 3 plants, 9498; Oct. '41, *D. J. Stouffer*, 8 plants, 9866.

9. *GEASTER CAMPESTRIS* Morgan, Am. Nat. 21: 1026. 1887
(FIG. 9)

Sporophore hypogeous with universal mycelium, small, button subglobose, 1-2 cm. across, some showing an umbilical scar, becoming superficial and expanded at maturity, then 1-3 cm. diameter, usual size about 2.5 cm. *Exoperidium* explanate to slightly revolute, split about two thirds way to middle. *Rays* 5-8, pliable, not hygroscopic, unequal, narrow, acute. *Fleshy layer* adnate, not rimose or splitting off from middle fibrous layer, bister to Mars brown. *Exterior* covered with a thin layer of blackish soil, held by the persistent, adnate mycelial layer. *Base* concave, some with a small umbilical scar but most not showing such a scar. *Endoperidium* sessile to usually short pedicellate, subglobose, 6-15 mm. wide by 5-10 mm. tall, densely covered with small brown granules or warts, asperate, wood brown under warts, dark gray to light drab when warts are included, easily rubbing off in handling, warts appearing like dark stippling with whitish bloom between them. *Peristome* small, circular, sulcate, acute, with 10-14 sulci of equal thickness and length, extending to apex of peristome, dark brown, seated in a definite depressed area, darker than the balance of the endoperidium, sepia color. *Gleba* sayal brown to snuff brown. *Columella* prominent, persistent in mature plant, globose. *Capillitium* simple, hyaline, solid, 2.8-4.2 μ thick. *Spores* globose, 5.6-6 μ diameter, usual size 5-6, uniguttulate, contents of spore tinted brown, semi-opaque in water. *Epispore* chestnut brown, verrucose.

Habitat: Gregarious in earth by sidewalk in city of Lincoln, Neb., or in open grassy prairies about Lincoln. Sept. 27, 1886.

Distribution: Vicinity of Lincoln, Neb., Sept. 27, 1886, C. E. Bessey, usually in open prairie.

The above is from type material. I soaked three plants for three hours in water without the least signs of any extension of the rays, so they cannot be called hygroscopic. The rays soften some but do not change in position. All three plants showed brown warts, which are rough or asperate when dry.

The type of *Geaster campestris* is from the open grassy prairies in the vicinity of Lincoln, Neb., hence its name, but all of our material from the Southwest is from shade or partial shade of desert vegetation and varies in many minor details from the type plants. The type is not at all hygroscopic, whereas our plants are more or less hygroscopic. The sizes, colors, stipes etc. vary much from the type and often from each other yet in spite of these differences we have included all these forms under the name *G. campestris*, since they intergrade so much that no fixed definite line of demarcation can be found. We are giving herewith for comparison the type description, the combined description from all our plants and the description from the Nogales specimens since they vary more than any others from the usual run of our material of this species. The Nogales plants differ more than any others and may deserve a varietal name but we doubt it, believing that the dry semi-arid environment of our climate here may have caused these changes from the type.

GEASTER CAMPESTRIS composite description from all collections
(FIG. 10)

Sporophore hypogeous with a universal mycelium, button small, subglobose, 1-2 cm. across, some showing an umbilical scar and some not, becoming superficial and more or less explanate at maturity, 1-4 cm. diameter when expanded, usual size about 2.5 cm. *Exoperidium* explanate, revolute or involute under (rarely over) the spore sac, split one half to two thirds of the way to middle.

Rays 5-12, acute, subequal, pliable to rigid, often subhygroscopic. *Fleshy layer* adnate, continuous, not rimose or cracking, bister, Mars brown to benzo drab. *Exterior* covered with dirt or

leaf debris, rarely peeling off and leaving the outer surface of the rigid fibrillose layer, naked. *Base* concave, sometimes with a small umbilical scar, but most of them not showing this scar. *Endoperidium* subsessile to short pedicellate, apophysis often present, subglobose, 6–18 mm. wide, covered with small, rough or asperate granules or warts seen only with a good hand lens but evident to the touch, wood brown, general color dark gray to light drab; often the granules rub off in handling. *Peristome* small, circular, sulcate, acute, with 10–20 sulci of equal thickness and length, extending to apex of peristome, light drab to dark brown, many seated in a depressed area but often not, usually darker than the endoperidium but often concolorous with it. *Gleba* sayal brown to chocolate brown to blackish brown. *Columella* globose, usually persistent in mature plants. *Capillitium* simple, hyaline, with thin or thick walls, threads 3–4 μ thick. *Spores* globose, 4.2–7 μ diameter, semi-opaque in water. *Epispore* subhyaline to chestnut brown, verrucose.

Habitat: Gregarious in this region in leaf debris under or at the edge of conifers and hardwood trees, rarely in open soil.

Distribution: ARIZONA, Flagstaff, 2–6–34, *W. H. Long*; 6 plants, 8979; Sabino Canyon area near Tucson, 6–4–35, 11 plants, 8769; Prescott, Peterson area, 99 plants, 7687; *W. H. Long & V. O. Sandberg* 4–34, 84 plants, 7689; 7 miles of Nogales, 2–19–34, 4 plants, 4867; 9–11–41, *W. H. Long & D. J. Stouffer*, 38 plants, 9629; 49 plants, 9635. Eagle District, Crook Nat. For., *D. J. Stouffer*, 3–26–47, 2 plants, 7863. New Mexico, Jemez Mts., *Ernest Knaebel*, 31 plants in Lloyd Myc. Coll. no. 52512; Eureka Lodge near Cuba, 8300 ft. elevation, *W. H. Long*, 37 plants, 7770; 9–13–33, 5 plants, 7767; 5–21–33, 19 plants, 8977; 8–13–39, 1 plant, 9069; 9–25–37, 11 plants, 9884; near Dulce, Stone Lake, 7600 ft., 6–25–34, *Ledru Savage & R. L. Turner*, 11 plants, 8022; Tres Montosas Mts. area near Magdalena, 10–11–34, *A. E. Frazier*, 3 plants, 7988; sandhill-juniper area 25 miles from Corona, *D. J. Stouffer*, 11–27–41, 2 plants, 9932; 12–15–41, 17 plants, 9963; 4–21–42, 9 plants, 10206; and 5 plants, 10201; 9–17–41, *W. H. Long & D. J. Stouffer*, 16 plants, 9740; Jicarilla, Lincoln County, 9–6–41, 4 plants, 9760; 9–16–41, 1 plant, 10028; 5–21–41, 6 plants, 9449; 20 miles N.W. Corona, 5–8–41, *D. J. Stouffer*, 8 plants, 9318; Cougar Mt. area, 4–21–40, *W. H. Long & D. J. Stouffer*, 7 plants, 8693; near Cougar Tank, 5–12–41, *D. J. Stouffer*, 2 plants, 9823; near Ranger Tank, 5–12–41, *D. J. Stouffer*, 4 plants, 9324; 9–15–41, *W. H. Long & D. J. Stouffer*, 1 plant, 9808; Corona area, *D. J. Stouffer*, 5–2–39, 20 plants, 8370; July, '40, 2 plants, 8708; Oct. '40, 3 plants, 9169; 4–4–41, 19 plants, 9267; 4–20–41, 3 plants, 9321; 4–1–41, 2 plants, 9269; April, '41, *W. H. Long & D. J. Stouffer*, 5 plants, 9510; 4–20–40, 10 plants, 9143; 9–6–41, 5 plants, 8566; 9–15–41, 10 plants, 9751; and 7 plants, 9728; 9–17–41, 9 plants, 9773; 4–17–42, 8 plants, 10068; April, '42, 4 plants, 10059. TEXAS, Denton, 12–23–07, *W. H. Long*, 2 plants, 2059; 7–12–03 (Long no. 1793), 15 plants in Lloyd Myc. Coll. no. 52514.

The collections from Eureka Lodge, and Dulce, New Mexico, and from the Peterson area near Prescott, Arizona, all high altitude localities, had numerous sessile to subsessile plants, making them approach *Geaster Drummondii*; also in some cases the plants had shed all of the outer mycelial layer and some were strongly hygroscopic, again making them resemble *G. Drummondii*, but other plants with these were typical *G. campestris*.

GEASTER CAMPESTRIS var. ? (FIG. 11)

Sporophore hypogeous with a universal mycelium; buttons small, subglobose, 6–10 mm. across, all showing an umbilical scar on base, becoming superficial at maturity, then a few are explanate but most of them not so, involute under base of sporè sac, 5–30 mm. across, usual size 1–1.5 cm. when fully expanded. *Exoperidium* mostly involute around base of sporè sac, a few revolute, split about half way to middle. *Rays* 6–10 rigid, subhygroscopic, unequal, acute. *Fleshy layer* adnate, continuous, not rimose when not weathered, minutely warty, then a stippled cinnamon drab to benzo drab, with age becoming smooth and chaetura black. *Exterior* covered with a layer of hyphae and leaf debris, held by the persistent mycelial layer. *Base* concave, with or without an umbilical scar. *Endoperidium* subsessile to usually short pedicellate, subglobose, 5–10 mm. wide by 5–12 mm. tall, usually with a distinct apophysis, densely covered with brownish rough granules or warts, light drab under warts, giving the surface a grayish stippled look. *Peristome* small, circular, sulcate, acute, with 12–16 sulci of equal thickness and length, extending to apex of peristome, some light drab but most of them hair brown to chaetura black, seated in a definite area darker than the balance of the endoperidium. *Gleba* mummy brown to blackish brown. *Columella* inevident or in some oblong to subglobose and persistent. *Capillitium* colored, fulvous, walls thin, 3–4 μ . *Spores* globose, verrucose, 5.6–7 μ diameter, opaque in water, uniguttulate. *Epispore* coarsely verrucose, wall subhyaline to chestnut brown.

Habitat: Gregarious in debris under catclaw and mesquite trees, in hot dry regions.

Distribution: On highway U. S. no. 89 from Nogales to Tucson.

10. GEASTER SMITHII Lloyd, Myc. Writ. 1: Geastrae, pp. 21 and 42. 1902 (FIG. 12)

Sporophore hypogeous in leaf debris, becoming superficial and expanded at maturity, then 1.5–4 cm. across. *Exoperidium* revolute, splitting to about the middle, often vaulted. *Rays* 7–13, subequal, acute, some splitting again at the tips, usually not hygroscopic but some plants slightly subhygroscopic, and involute under the spore sac, when dry. *Fleshy layer* thin, adnate, often rimose and peeling, lead color to ferruginous, farinose when fresh. *Exterior* covered with leaf debris by adnate mycelial layer which often peels off at the tips, these rays then becoming involute, exposed under side of the naked rays light tan. *Base* concave, rarely umbilicate. *Endoperidium* short pedicelled, subglobose to pyriform to urceolate, 5–18 mm. across, usually chestnut brown but one collection from Arizona pallid tan, papyraceous. *Peristome* flattened-conical, seated in a very definite depressed area, concolorous or often darker than the endoperidium, regularly sulcate-striate, sulci unequal, 20–30, many sulci short and not reaching top of peristome. *Gleba* chestnut brown to blackish brown. *Columella* inevident. *Capillitium* subhyaline to fulvous, thinner than the spores. *Spores* dark fuliginous, almost opaque, globose, 4–5 μ diameter. *Epispore* verruculose.

Habitat: Gregarious in leaf debris usually under *Juniperus monosperma*.

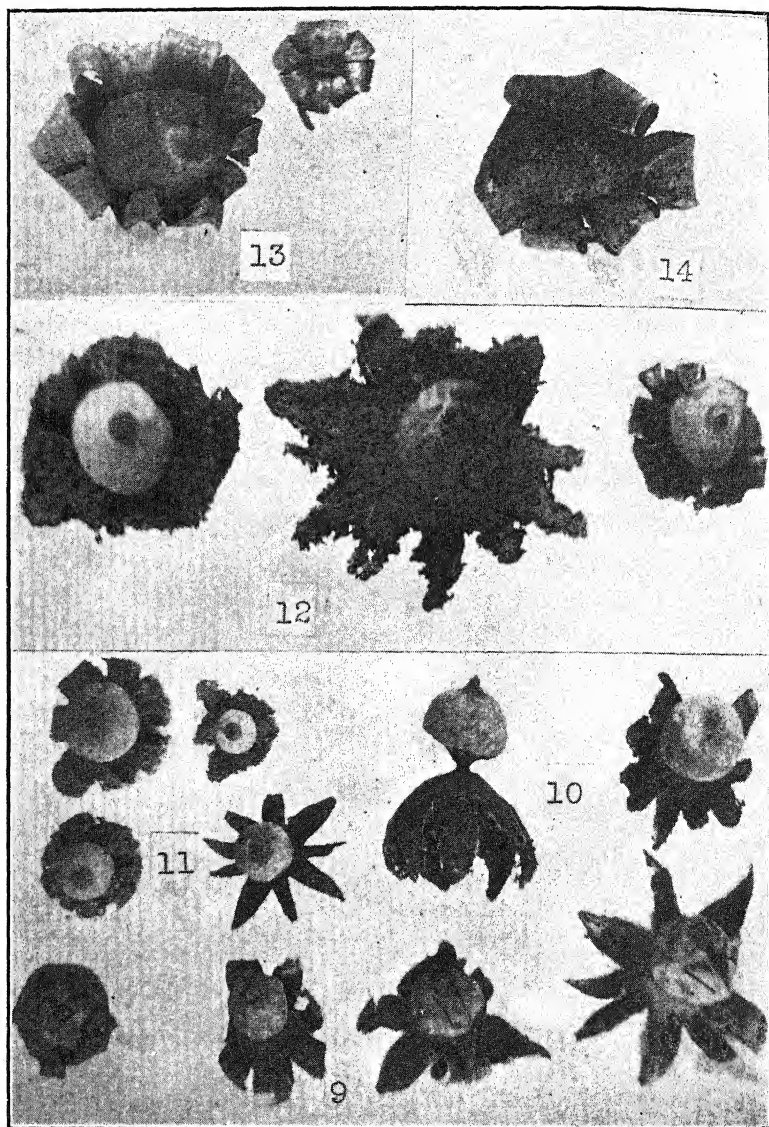
Distribution: ARIZONA, Sabino Canyon area, 9–28–39, *W. H. Long*, 1 plant, 9144; Safford, 9–9–40, *W. H. Long & D. J. Stouffer*, 1 plant, 10024; 9–9–41, 4 plants, 9706; near Young in Tonto Nat. For., August, '44, *N. F. Noecker & L. W. Robeson*, 8 plants, 7970. NEW MEXICO, Corona area, 4–17–42, *W. H. Long & D. J. Stouffer*, 61 plants, 10069; near Atkinson Ranch, 9–21–42, 46 plants, 10116; Tres Montosas Mts. near Magdalena, 10–11–34, *A. E. Frazier*, 1 plant, 8892; Corona area, 9–4–41, *W. H. Long & D. J. Stouffer*, 24 plants, 9505; 9–14–41, *W. H. Long & D. J. Stouffer*, 8 plants, 9764; 2–19–40, *D. J. Stouffer*, 2 plants, 9286; 4–6–43, 2 plants, 10357; 25 miles N.W. of Corona, 4–20–40, 2 plants, 9287; 1–14–42, 83 plants, 9993; 9–17–41, *W. H. Long & D. J. Stouffer*, 91 plants, 9777; Corona area, 7000 feet elevation, 2–28–40, *D. J. Stouffer*, 2 plants, 8476; 4–4–41, 11 plants, 9265; Oct. '41, 20 plants, 9861, in sand hills along Gran Quivera road, Sept. '41, 7 plants, 9843; Oct. '41, 16 plants, 9865; April, '40, *W. H. Long & D. J. Stouffer*, 10 plants, 9096; July, '40, 13 plants, 8724; 4–1–40, 15 plants, 8726; 9–15–41, 19 plants, 9662; near Ranger Tank, 9–5–41, 4 plants, 9802; sandhill-juniper dunes about 20 miles from Corona, 9–17–41, 4 plants, 9738; Cedarvale area, Pinos Mts., Nov. '40, *D. J. Stouffer*, 7 plants, 9216; Atkinson sandhill pasture, 25 miles from Corona, 12–15–41, 4 plants, 9966.

11. GEASTER MAMMOSUS (Chev.) Fr. Syst. Myc. 3: 17. 1829
(FIG. 13)

Sporophore hypogeous with a universal mycelium, becoming superficial, and expanded at maturity, then 1-4 cm. across. *Exoperidium* revolute when wet, splitting nearly to the base. Rays 8-12, unequal, acute, rigid, strongly hygroscopic, more or less involute over the entire spore sac when dry, dirty white to light brown externally. *Fleshy layer* adnate, continuous, not rimose, clove brown to chaetura black in age. *Exterior* at first covered with a thick mycelial layer of dirt and hyphae, deciduous and leaving the outer surface of the fibrillose layer clean of dirt and a dingy white, white in the button stage. *Base* slightly concave when rays are expanded, not umbilicate. *Endoperidium* subglobose, sessile, 1-1.5 cm. across, Rood's brown, slightly furfuraceous, partially smooth in age, globose to depressed-globose. *Mouth* definite with a silky peristome, conic, mouth parts usually a darker color than balance of the peristome. *Gleba* blackish brown to chaetura black. *Columella* short, cylindrical, pointed. *Spores* globose, light brown, 3-5 μ diameter. *Epispore* brownish, minutely verrucose.

Habitat: Solitary or gregarious in small groups in partial shade of piñons and junipers.

Distribution: ARIZONA, V. T. Ranch near old airfield, 8800 ft., *W. H. Long*, 10 plants, 7841; Grand Canyon, Aug. '34, *Dr. R. B. Street*, 1 plant, 8968; Blue River area, Eagle District, Crook Nat. For., 3-24-47, *D. J. Stouffer*, 13 plants, 11445. NEW MEXICO, Corona area, 7000 ft., 4-16-42, *W. H. Long*, 7 plants, 10058; 4-17-42, 11 plants, 10078; 9-6-41, 16 plants, 9568; 4-14-42, 38 plants, 10236; 9-15-41, 3 plants, 9726; Sept. '40, *D. J. Stouffer*, 8 plants, 9130; 11-7-40, 6 plants, 9212; 5-25-41, 8 plants, 9432; 7-5-41, *Jack Porter*, 11 buttons, 9366; 7-16-41, 11 plants, 9539; 9-17-41, *W. H. Long & D. J. Stouffer*, 56 plants, 9780; 9-15-41, 13 plants, 9752; 9-17-41, 18 plants, 9742 and 14 plants, 9660; 9-14-41, 75 plants, 9768; 4-21-42, 37 plants, 10119; Atkinson Ranch, 25 miles from Corona, 12-15-41, *D. J. Stouffer*, 3 plants, 9965; near Cougar Tank, 5-28-41, 5 plants, 9424; near Mulkey Ranch, 20 miles N.W. of Corona, April, '41, 13 plants, 9319; Parida Canyon near Willard, 11-11-40, 1 plant, 9202; 11-29-40, 1 plant, 9202; 11-29-41, 11 plants, 9938; near Vaughn, 6-15-41, 5 plants, 9419; Lincoln County, Jicarilla, 9-16-41, *W. H. Long & D. J. Stouffer*, 48 plants, 9761; Deming, 10 miles west, 9-9-41, 1 plant, 9832; Tres Ritas, Amole Canyon, 8200 ft., Oct. '10, *W. H. Long*, 45 plants, 8864; La Junta Canyon, Oct. '14, 14 plants, 8863; and 12 plants, 5242; Corona area, 4-20-42, *W. H. Long*, 110 plants, 10219; 9-6-41, 10 plants, 11441; 4-21-40, *W. H. Long & D. J. Stouffer*, 142 plants, 8737; 4-21-40, *W. H. Long & D. J. Stouffer*, 32 plants, 7829; *D. J. Stouffer*, 2-28-40, 27 plants, 8481; near Cougar Mt., 7100 ft., May, '40, 13 plants, 8825; 4-21-40, *W. H. Long & D. J. Stouffer*, 27 plants, 8734; Oct. '40, *D. J. Stouffer*, 21 plants, 9173; 4-20-40, *W. H. Long & D. J. Stouffer*, 13 plants, 8702. TEXAS, Denton, *W. H. Long*, 12-23-07, 15 plants, 2028.



FIGS. 9-14, $\times 1$. *Geaster*; 9, *G. campestris* from type material, 4 plants; 10, *G. campestris* usual form, 2 plants; 11, *G. campestris*, form near Nogales, 4 plants; 12, *G. Smithii*, 3 plants; 13, *G. mammosus*, 2 plants; 14, *G. xylogenus*, 1 plant.

12. *Geaster xylogenus* sp. nov. (FIG. 14)

Sporophoro lignicolo. *Exoperidio* revoluto, coriaceo, subhygrometrico, usque ad centrum in 8 laciniis, involutis, acuminatis fisso. *Endoperidio* stipitato, toto evanescenti. *Stipite* crasso, subligneo, 5 mm. crasso, 10 mm. lato, 2 mm. alto. *Capillitio* subhyalino vel fusco, 5-6 μ lato, simplici. *Sporis* globosis, 3.2-5 μ diametro, verrucosis.

Sporophore epigeous, buttons not found but apparently they were acute, judging from the acuminate tips of the exoperidium, becoming expanded at maturity. *Exoperidium* revolute, rigid, coriaceous, subhygroscopic, splitting nearly three fourths the way to the center into 8 segments, deeply concave below, dome-shaped above. *Rays* unequal, recurved, with strongly involute acuminate curled tips, 4-5 cm. long, faintly longitudinally striate. *Exterior* naked, smooth, pecan brown. *Fleshy layer* adnate but peeling off above, chestnut brown, thin. *Endoperidium* short stipitate, deciduous (only fragments left), brittle, breaking away at base, Mars brown. *Sterile base* none. *Stipe* stout, subligneous, 5 mm. thick by 10 mm. wide by 2 mm. tall. *Gleba* liver brown, but little remaining. *Columella* inevident. *Capillitium* subhyaline to light fuliginous, thicker than spores, 5-6 μ thick, unbranched. *Spores* globose, 3.2-5 μ , usual size 4.2 μ . *Epispore* dark brown, verrucose.

Habitat: Solitary, growing on an old rotting, prostrate pine log (*Pinus ponderosa*) in shade of Gambel oak (*Quercus gambelii*).

Distribution: NEW MEXICO, Sandoval County in lower part of Senorito Canyon about 6 miles from Cuba, elevation 6400 ft., W. H. Long, 9-26-37, no. 11028 *Type* (1 plant).

This plant is peculiar in that it has a deciduous endoperidium similar to the genus *Terrostella* but does not have the sterile base of that genus, nor does it have the persistent subligneous columella of a *Trichaster*. We are therefore leaving it under the genus *Geaster*.

13. *GEASTER ARENARIUS* Lloyd, Myc. Writ. 1: Geastrae 28. 1902 (FIG. 17)

Sporophore hypogeous in leaf debris and soil, becoming superficial and expanded at maturity, then 1.5-2.5 cm. across. *Exoperidium* rarely explanate or revolute, usually involute around base of spore sac, splitting nearly to middle or only to middle. *Rays* 8-12, very unequal, some explanate to revolute but mostly involute around the spore sac, weakly subhygroscopic, thin, acute. *Fleshy layer* thin, adnate, not rimose, clay color to sayal brown. *Exterior*

covered with leaf debris and dirt, not peeling off. *Endoperidium* globose to subglobose, pallid mouse gray to dingy white, sessile to subsessile, rarely with a short pedicel, very thin, brittle and papery. *Peristome* very definite in a depression, much darker than the endoperidium, deep mouse gray to dark mouse gray, conic, often fibrillose. *Gleba* seal brown. *Columella* globose. *Capillitium* light brown, walls thin, lumen large, with brown walls, 3–4 μ thick. *Spores* globose, 3.5–4.2 μ in diameter. *Epispore* verruculose.

Habitat: Gregarious in leaf debris under mesquite and catclaw.

Distribution: ARIZONA, Sabino Canyon area, 2–20–34, *W. H. Long & V. O. Sandberg*, 6 plants, 7609; 11–11–36, 4 plants, 8897; 6–4–38, *W. H. Long*, 49 plants, 8314; 11–10–38, 12 plants, 8256; 6–21–38, 77 plants, 9263; 9–28–39, 23 plants, 8392; 7 miles from Nogales on highway 84, 11–23–33, *W. H. Long & V. O. Sandberg*, 1 plant, 7853; 2–29–34, 21 plants, 8768; 11–13–36, 23 plants, 8846; 6–4–38, 31 plants, 8304; 9–11–41, *W. H. Long & D. J. Stouffer*, 55 plants, 9627; and 81 plants, 9634; 63 plants, 9696; Mt. Mingus, Prescott Nat. For., 5–18–34, *W. H. Long*, 2 plants, 8813. NEW MEXICO, Jornada Exp. Range, 9–8–41, *W. H. Long & D. J. Stouffer*, 1 plant, 9708; 10 miles west of Deming, *W. H. Long*, 4–24–42, 9 plants, 10062. TEXAS, Denton, 1–10–03, *W. H. Long*, no. 1788, 16 plants, Lloyd Myc. Coll. no. 52513, as *Geaster asper*. This collection is very much like the plants collected 7 miles from Nogales, Arizona.

14. GEASTER TOMENTOSUS Lloyd, Myc. Writ. 5: 818. 1919
(FIG. 18)

Sporophore epigeous with fibrillose basal mycelium, more or less expanded at maturity, then 1.5 cm. across, buttons subglobose. *Exoperidium* flaccid, more or less revolute, opening about one half to center, leaving the base strongly saccate. *Rays* subequal, 6–8, acute, not hygroscopic. *Fleshy layer* pecan brown, adnate, not rimose or flaking off. *Exterior* covered with a matted tomentum, persistent unless worn away by age or handling, light pinkish cinnamon. *Endoperidium* subglobose, sessile, saccate, light drab, about 1 cm. in diameter, with a prominent peristome. *Peristome* circular with a border, fibrillose, about 5 mm. in diameter, darker than the endoperidium, mouse gray, of different texture than the surrounding tissue. *Mouth* subconical. *Gleba* blackish brown to black. *Columella* slender, linear. *Capillitium* brown, not branched. *Spores* globose, dusky brown, semi-opaque, 4.2–4.5 μ . *Epispore* verrucose.

Habitat: Gregarious to cespitose, on ground in open pine woods.

Distribution: TEXAS, Houston, *George L. Fisher*, no. 26, 10-30-18, 7 plants including 5 buttons in Lloyd Myc. Coll. no. 54721 **Type**; in woodland pasture, *George L. Fisher*, no. 28, 10-30-18, 2 buttons in Lloyd Myc. Coll. no. 4800; on ground (*Fisher* no. 19), 3 buttons, in Lloyd Myc. Coll. no. 54720.

The opened plants resemble very much a small form of *Geaster saccatus* as noted by Lloyd (1919) whereas the buttons with their fibrillose basal roots are much like an unopened *Lycoperdon*. This species has not been reported since the above records as far as we know.

15. *GEASTER MINIMUS* Schw. Syn. Fung. Car. p. 58, n. 327. 1822
(FIGS. 15-16)

Sporophore hypogeous in leaf debris with universal mycelium, becoming superficial and expanded at maturity, then 1-4 cm. *Exoperidium* revolute, split to about the middle. *Rays* 8-12, subequal, acute, often recurved till they are more or less vertical, tips occasionally involute around edge of dome. *Fleshy layer* adnate, rarely cracking, Mikado brown to light pinkish cinnamon. *Exterior* covered with debris, shaggy from the adhering fragments, persistent. *Base* concave to vaulted, not umbilicate. *Endoperidium* pedicellate, pedicel short, subglobose to ovate to often elongated pear-shaped, 3-10 mm. diameter. *Mouth* definite, grooved, with a silky peristome, usually seated in a depression bordered by a ring, peristome lighter or darker or concolorous with endoperidium. *Gleba* seal brown. *Columella* subglobose. *Capillitium* hyaline. *Spores* globose, 5.4-5.5 μ in diameter. *Epispore* slightly verruculose but some appearing smooth even under oil immersion lens.

Habitat: Gregarious under conifers and hardwoods in the leaf debris.

Distribution: ARIZONA, Sabino Canyon area near Tucson, 6-4-38, *W. H. Long*, 49 plants, 8314; Young in Tonto Nat. For., August, '38, *N. L. Noecker & L. W. Robeson*, 15 plants, 8906; Safford, 9-9-41, *W. H. Long & D. J. Stouffer*, 2 plants, 2703; Grand Canyon, Arizona, 1930, Dr. R. B. Street, 3 plants, 7889; Flagstaff and vicinity, 8300 feet, 7-4-36, *W. H. Long*, 1 plant, 9374; 9 plants, 7802; 4 plants, 7695; 12 miles from Flagstaff, 5-2-33, 6 plants, 8869. NEW MEXICO, Corona area, 4-20-40, *W. H. Long & D. J. Stouffer*, 230 plants, 8705; 4-15-42, 11 plants, 10234; 4-21-40, 8 plants, 8740; September, '40, *D. J. Stouffer*, 5 plants, 9126; 9-17-41, *W. H. Long & D. J. Stouffer*, 2 plants, 10008; 20 miles N.W. Corona, 1-2-41, *D. J. Stouffer*, 6 plants, 9247; 7-23-41, 2 plants, 9553; Lincoln County, Jicarilla, 5-6-41, 27 plants, 9315; 4-1-41, *D. J. Stouffer*, 6 plants, 9270; 5-19-41, 3 plants, 9447; 2-14-40, 14 plants, 9251; 9-4-41, *W. H. Long & D. J. Stouffer*, 6 plants, 9504; 9-17-41, 5 plants, 9738; 5 plants, 9774; 9-18-41,

8 plants, 9750; 4-16-42, 5 plants, 10209; 4-17-42, 28 plants, 10076; Atkinson Ranch, 4-21-42, 33 plants, 10203; Ranger Tank, D. J. Stouffer, 9-5-41, 17 plants, 9528; Cougar Tank, 10 plants, 9397; Corona area, 1-24-41, 12 plants, 9255; Mulkey Ranch, 11-7-40, 43 plants, 9251; Jicarilla, Lincoln County, 2-15-41, 1 plant, 9257; 7-18-41, 2 plants, 9544; 9-16-42, W. H. Long & D. J. Stouffer, 31 plants, 9759; Red Cloud Picnic Grounds, 4-16-42, 1 plant, 10242; 40 plants, 10228; Jicarilla, Lincoln County, 9-15-41, 34 plants, 9671; Willard in Parida Canyon, 11-29-41, D. J. Stouffer, 5 plants, 9943; Tres Montosas Mt. area near Magdalena, 10-16-34, A. E. Frazier, 17 plants, 8890; White Sands National Monument, 4-12-42, W. H. Long, 1 plant, 10115; Pecos, 11-25-12, 67 plants, 8862; 1916, 6 plants, 8865. TEXAS, Denton, Dec. '02, W. H. Long, no. 1785, 4 plants, Lloyd Myc. Coll. no. 23009; 12-23-07 (Long no. 2051), 2 plants, Lloyd Myc. Coll. no. 23022; 10-10-07, W. H. Long, 2 plants, 2029; 12-23-07, 4 plants, 2051.

A comparison was made with the type material of *Geaster juniperinus* and it was found that this species is only a larger and darker form of *G. minimus* and not a form of *G. coronatus* as suggested by some writers. Also we do not believe that *G. coronatus* is a synonym of *G. minimus*. Johnson (1929) makes very clear the differences between the two species and her reasons for not combining them seem valid. The assumption that certain plants belong to the same species because they have been found growing from the same mycelial mat is not proof that they are the same. We have found *G. Smithii* and *G. fornicatus*, side by side from the same mycelial substratum, and yet no one could claim they are the same species.

16. *GEASTER CORONATUS* (Schaeff.) Schroet. Pilze Schles. p. 702.
1889 (FIG. 19)

Sporophore hypogeous in leaf debris with a universal mycelium, becoming superficial and fornicate at maturity, then 1-1.5 cm. across at base. *Exoperidium* revolute, bending strongly backward and downward, usually splitting to beyond the middle. *Rays* 4-5, broad, subequal, tips attached to the fornicate mycelial layer, not hygroscopic, but rigid. *Fleshy layer* Vandyke brown to burnt umber in age, then shining and peeling off and leaving a naked surface light buff to dingy white. *Exterior* mycelial layer is stripped off from the fibrous layer when evagination occurs and remains as a more or less hollow cup attached to the tips of the rays. *Endoperidium* pedicellate, ovate to oblong, attenuate at base, with a prominent apophysis, plum colored (bone brown), 5-10 mm. broad by 8-12 mm. tall. *Mouth* definite, prominently raised,

seated in a depressed area with a definite silky zone, bounded by a ridge. *Peristome* markedly lighter in color than the endoperidium, sayal brown. *Gleba* seal brown. *Columella* slender or in-evident. *Capillitium* pale brown, 2-5 μ thick, rarely branched. *Spores* globose, 4-4.5 μ in diameter. *Epispore* distinctly warted.

Habitat: Gregarious in leaf debris in a mixed stand of cork-bark fir (*Abies arizonica*), Douglas fir (*Pseudotsuga taxifolia*) and aspen (*Populus tremuloides*) at an elevation of 9000 feet.

Distribution: ARIZONA, Mt. Graham in Crook Nat. For., 5-8-47, D. J. Stouffer, 30 plants, 11458.

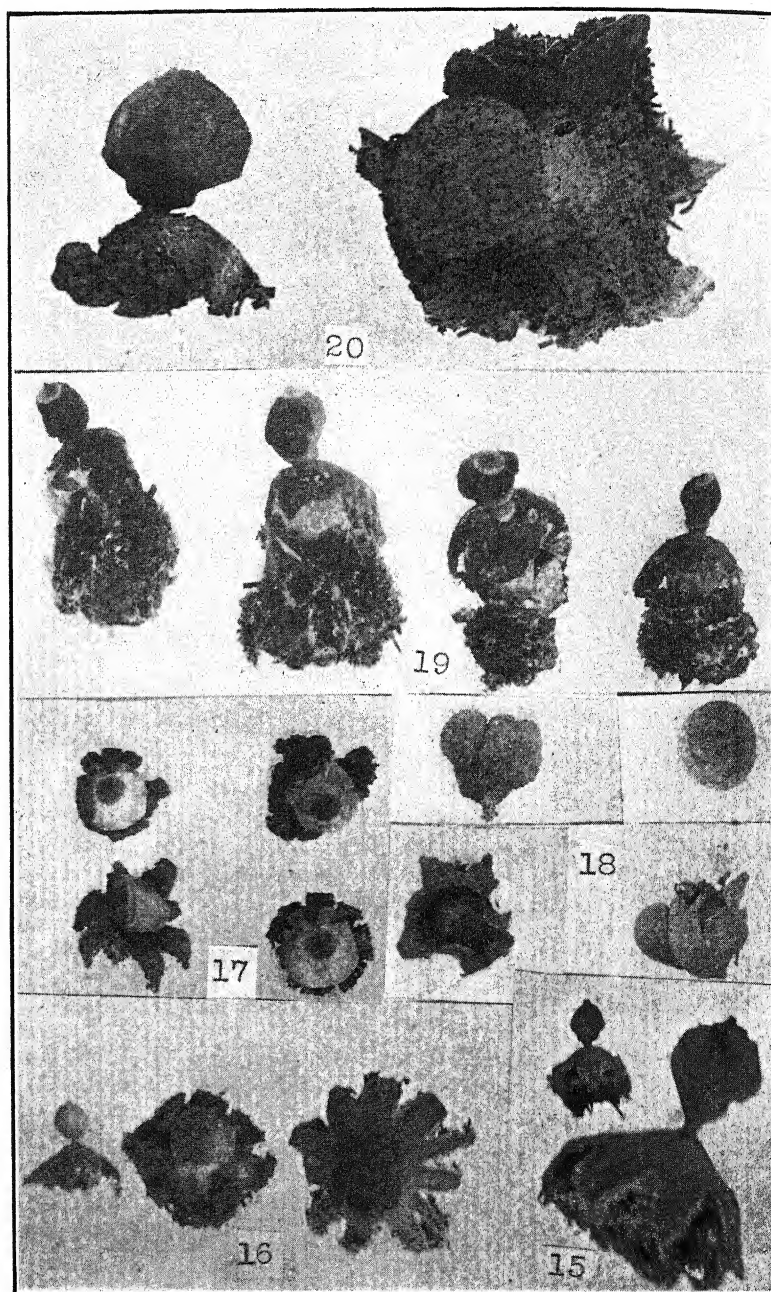
These plants apparently are smaller than normal. The spore sacs and peristomes are very uniform in color and the fleshy layer usually peels off in weathering. This is the first time this beautiful little species has been found in our territory.

17. GEASTER HIERONYMI Henn. Hedw. 36: 211. 1897 (FIG. 20)

Sporophore hypogeous in leaf debris, becoming superficial and expanding at maturity, then 3-7 cm. across; buttons globose. *Exoperidium* revolute, often involute, usually splitting to about the middle. *Rays* 6-8, rarely 10, revolute or sometimes partially involute beneath the spore sac, acute. *Fleshy layer* bay brown or darker, thin, adnate, often peeling off and leaving the fibrous layer chestnut brown. *Exterior* covered with debris. *Base* concave, not umbilicate. *Endoperidium* short pedicellate, the stalk often strongly flattened with a prominent apophysis, depressed-globose, obovate to urceolate, harshly asperate with prominent black spicules, dark brown to grey-brown to silvery brown, much like the spore sac of a *Myriostoma*, 1.5-3 cm. across. *Mouth* indefinite, naked, short, slightly conical, often fimbriate with age. *Gleba* sepia. *Columella* large, globose to conical. *Capillitium* light brown, 3-5 μ thick. *Spores* 2.9-4.5 μ diameter. *Epispore* minutely verruculose.

Habitat: Gregarious in leaf debris of *Juniperus monosperma*.

Distribution: NEW MEXICO, Corona area, 4-17-42, W. H. Long & D. J. Stouffer, 32 plants, 10071; 9-17-41, 1 plant, 10006; 9-17-41, 126 plants, 9779; 4-4-41, D. J. Stouffer, 6 plants, 9285; 6-22-41, 1 plant, 9343; Oct. '40, 20 plants, 9175; 12-29-40, 10 plants, 9239; 1-16-41, 11 plants, 9250; 9-4-41, W. H. Long & D. J. Stouffer, 34 plants, 9494; 9-14-41, 4 plants, 9766; D. J. Stouffer, 4-1-41, 12 plants, 9268; 3-7-41, 13 plants, 9826;



Cougar Mt. area, 11-8-40, 10 plants, 9284; 30 miles from Corona, 3-31-41, 15 plants, 9283; Corona area, 5-25-41, 35 plants, 9809; 1-14-42, 37 plants, 9992.

18. *GEASTER LIMBATUS*, (black European form), Fr. Syst. Myc. 3: 15. 1829 (FIG. 21)

Geaster limbatus Fries var. *pacificus* Morse, Mycologia 33: 139-142. 1941

Sporophore hypogeous in leaf debris with both a universal and basal mycelium, becoming superficial and expanded at maturity, then 2-6 cm. across. *Exoperidium* revolute, splitting to the middle. Rays broad, 7-12, acute, subequal, not hygroscopic, more or less vaulted, or some involute under spore sac, rays often splitting again at the tips which are occasionally involute. *Fleshy layer* adnate, thick, rarely splitting and flaking off, usually of same color as the spore sac, when not weathered, natal brown to avellaneous on some freshly expanded plants, then a lighter color than the spore sac. *Exterior* covered with leaf debris, deciduous only after long weathering. *Base* concave, often showing a strong umbilical scar from the basal mycelial strand. *Endoperidium* pedicellate, pedicel often flattened and with a pronounced apophysis, subglobose to depressed-globose, natal brown often with a white bloom, 10-25 mm. across, rarely weathering to dingy white. *Mouth* definite, often somewhat depressed, fibrillose, usually concolorous with the endoperidium. *Gleba* seal brown. *Columella* slender or inevident. *Spores* globose, 4-5.5 μ in diameter. *Epispore* dark brown, strongly verrucose.

Habitat: In leaf debris under *Juniperus monosperma* in our region.

Distribution: NEW MEXICO, Corona area, 9-5-41, W. H. Long & D. J. Stouffer, 9 plants, 9667; 25 miles N.W. of Corona, 11-27-41, D. J. Stouffer, 8 plants, 9931; Parida Canyon near Willard, 11-11-41, 4 plants, 9942; Corona area, 4-17-42, W. H. Long, 27 plants, 10105; 9-17-41, W. H. Long & D. J. Stouffer, 59 plants, 9763; Atkinson Ranch, 25 miles from Corona, 12-15-41, D. J. Stouffer, 37 plants, 9961; Corona area, 9-17-41, W. H. Long & D. J. Stouffer, 67 plants, 9778; 20 miles north of Corona, 4-2-41, D. J. Stouffer, 13 plants, 9518; Cougar Mt., 9-15-40, 7 plants, 9165; Corona area, July, '40, W. H. Long & D. J. Stouffer, 33 plants, 8722; 4-20-40, 61 plants, 8725; 12-29-40, D. J. Stouffer, 31 plants, 9238; 4-17-40, W. H. Long &

FIGS. 15-20, $\times 1$. *Geaster*; 15, *G. minimus* (type of *G. juniperinus* from Herb. Morgan), 2 plants; 16, *G. minimus*, usual form, 3 plants; 17, *G. arenarius*, 4 plants; 18, *G. tomentosus*, 4 plants; 19, *G. coronatus*, 4 plants; 20, *G. Hieronymi*, 2 plants.

D. J. Stouffer, 51 plants, 8723; Indian Springs, 7700 ft., 9-5-41, *D. J. Stouffer*, 1 plant, 9186.

GEASTER LIMBATUS (light colored American form, FIG. 22)

The black European form of *G. limbatus* is very common under *Juniperus monosperma* in the Corona region but we have not found it outside of this territory; although we have seen three plants of the same species from Berkeley, California, where it is again found under junipers but not *J. monosperma*. Miss Morse (1941) described it as a new variety of *Geaster limbatus*, but we do not think that it deserves a varietal name judging from the three plants we have seen from her. Under our distribution we have listed the black form separately from the typical form in the three states discussed.

The pale form is lighter in color than the typical form and much more varied in the colors of the fleshy layer and spore sac and differs also in other minor features. Its colors vary from fawn color to Hays brown or pale to pinkish buff for the fleshy layer, whereas spore sac or endoperidium varies in individual collections from eucere-drab to drab gray to plain drab; in old weathered plants often dingy white. Except for the colors, the characters are fairly constant. We are therefore holding all these color variations under the same name *Geaster limbatus*, but have described the black form in our technical diagnosis and have given photographs of both.

We have found only the black form under junipers whereas the lighter colored form is found under a variety of conifers and hardwoods, and, as our records show, it is much more widely distributed. We have compared our black form with a specimen from Dr. Höllós of Hungary and the two are comparable in every major detail.

Distribution: ARIZONA, Ft. Valley Exp. Station near Flagstaff, under pines, 7-1-15, *W. H. Long*, 1 plant, 5406; Mt. Mingus, Prescott Nat. For., 7000 ft., 5-18-34, 2 plants, 8812; Prescott area, 5-15-33, 8 plants, 8870; Chiricahua Nat. Monument, Bonito Canyon, under *Cupressus arizonica*, 11-25-33, *W. H. Long & V. O. Sandberg*, 36 plants, 7856; Grand Canyon, Arizona, August, '30, *Dr. R. B. Street*, 1 plant, 8967; Workman Creek, Tonto Nat. For. in hardwood duff, 9-27-46, *D. J. Stouffer*, 1 plant 11405; Eagle District, Crook Nat. For., 3-24-47, 11454. NEW MEXICO, Pecos, 5-10-16, *W. H. Long*, 7 plants, 5682; Tres Ritas, Penasco Canyon, 11-5-11, 13

plants, 8857; Gila Nat. For. Leg. Page, 1 plant, 8160; *W. H. Long*, 1 plant, 10011; Silver City, 7500 ft., 7-23-33, 5 plants, 8965; 10 plants, 8858; Pecos Town, 2 plants, 8867; Corona area, 9-5-41, *W. H. Long*, & *D. J. Stouffer*, 8 plants, 9533; Red Cloud Picnic Grounds, 4-16-42, 1 plant, 10241; Indian Springs, 8-31-41, *D. J. Stouffer*, 2 plants, 9818; Corona area, 9-6-41, 13 plants, 9569; 4-16-42, *W. H. Long* & *D. J. Stouffer*, 10 plants, 10053; 4-20-42, 1 plant, 10226; 4-15-42, 5 plants, 10235; 7 plants, 10240; 4-21-40, 8 plants, 8741; 9-17-41, 12 plants, 10010; Sept. '40, *D. J. Stouffer*, 7 plants, 9828; 72 plants, 9168; 9-17-41, 28 plants, 9778; Tejaso Canyon near Albuquerque, Oct. '16, *W. H. Long*, 1 plant, 5631; 6-25-22, 3 plants, 8766; 1916, 1 plant, 5580; Corona area, March, '39, *D. J. Stouffer*, 5 plants, 8465; 4-28-41, 2 plants, 9327; Jicarilla, Lincoln County, 5-6-41, 6 plants, 9316; 9-16-40, *W. H. Long* & *D. J. Stouffer*, 3 plants, 9762; Mimbres Valley, 5-22-35, *W. H. Long*, 2 plants, 9025; Tres Montosas Mts. near Magdalena, 10-11-34, *A. E. Frazier*, 1 plant, 8893; 20 miles N.W. of Corona, 7-23-41, *D. J. Stouffer*, 5 plants, 9550; Gran Quivera Road, 4-21-42, *W. H. Long* & *D. J. Stouffer*, 9 plants, 10107; Atkinson Ranch, 4-21-42, 7 plants, 10205.

19. *GEASTER SACCATUS* Fr. Syst. Myc. 3: 16, form MINOR Lloyd
Myc. Writ. 1: 111. 1902 (FIG. 23)

Sporophore hypogeous, in black soil becoming superficial and expanded at maturity, then 1.5-2 cm. across. *Exoperidium* semi-revolute, flaccid, not hygroscopic, splitting to middle or slightly beyond. Rays 6-7, broad, subequal, acute, not long pointed, saccate to shallowly saccate. *Fleshy layer* adnate, not rimose, pecan brown when not weathered, often aging to burnt umber. *Exterior* with a small amount of soil often attached. *Base* flat, concave or convex, some with a slight umbilical scar. *Endoperidium* sessile, papyraceous, not flaccid in any plants, saccate to subsaccate, subglobose, 8-10 mm. across, cinnamon drab to benzo brown, with a circular ridged peristome. *Peristome* prominent, concolorous or slightly lighter than the surrounding tissue, of a different texture, *Mouth* low, conical. *Gleba* sepia to seal brown. *Columella* globose. *Capillitium* hyaline, 4-5 μ thick, unbranched. *Spores* globose, 4-4.5 μ in diameter. *Epispore* verruculose.

Habitat: In black soil under barbed wire fences and under trees on Hickory Creek.

Distribution: TEXAS, Denton, *W. H. Long*, no. 7470, 1 plant in Lloyd Myc. Coll. no. 24991 as *Geaster* sp.; Nov. '02 (Long no. 1815), 2 plants in Lloyd Myc. Coll. no. 52585; 1-10-03 (Long no. 7474), 3 plants in Lloyd Myc. Coll. no. 22652; 1-12-05 (Long no. 7478), 4 plants in Lloyd Myc. Coll. no. 51283; 10-10-07 (Long nos. 2028 & 2068), 10 plants in Lloyd Myc. Coll. no. 52469 (named *Geaster arenarius* but certainly not that species); Austin, 1900, *W. H. Long*, 1 plant, 11198; 3-2-03, *W. H. Long* & *A. M.*

Ferguson, 3 plants, 11195; and 6 plants, 11194; Quitman, W. H. Long, 3 plants, 4541.

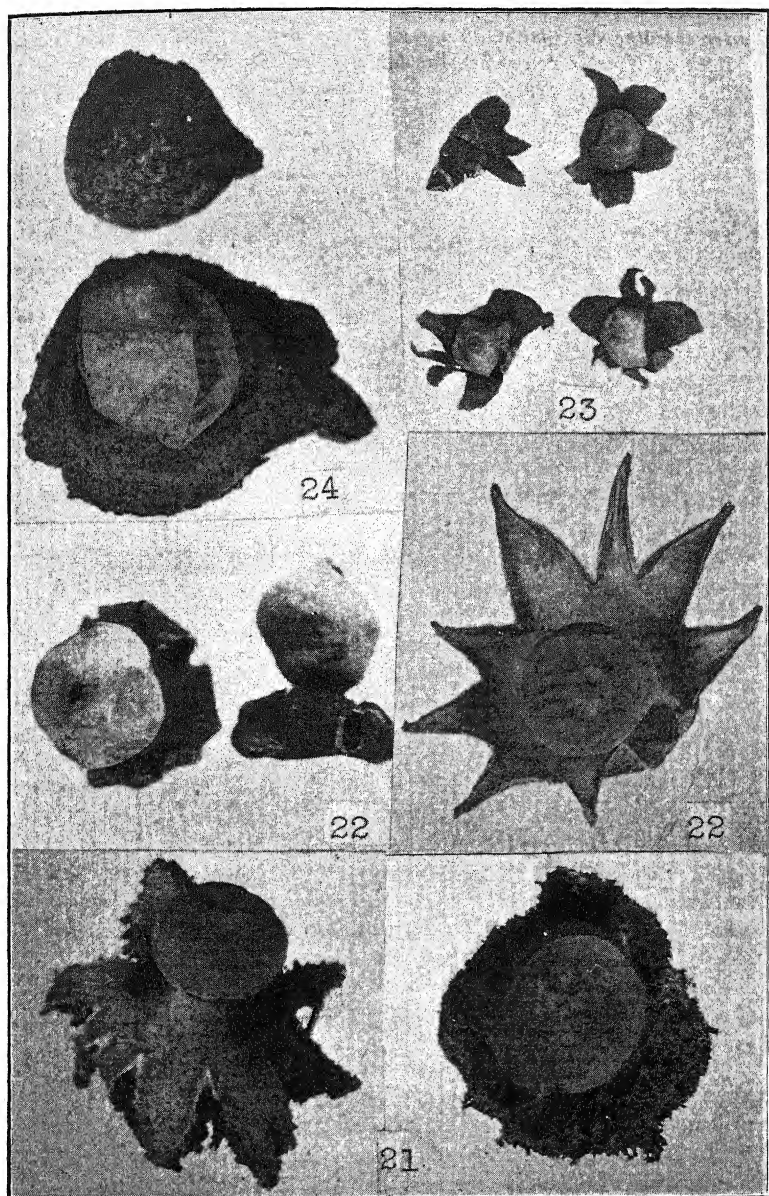
Farmers in plowing their prairie fields in the vicinity of Denton, Texas, during 1900-1910, where barbed wire fences were used, began plowing on the perimeter of the fields, going round and round toward the center. The dirt from the first furrow next to the fence usually fell on the area under the fence, thereby covering all trash, green and dead vegetation. The green buried grass and weeds of course will die and soon rot, as this process is repeated year after year on both sides of the fence, these fence rows in time will have more humus and decaying vegetable matter than the open fields; this produces good feeding grounds for certain fungi. This explains the habitat listed as "in black soil under barbed wire fences."

20. *GEASTER TRIPLEX* Jungh. Tidjschr. v. Natuurl. Ges. 7: 287. 1840 (FIG. 24)

Sporophore hypogeous in leaf debris, becoming superficial and expanded at maturity, then 3-8 cm. across, button globose or acute. *Exoperidium* explanate to revolute, rigid but not hygroscopic, splitting to or beyond the middle. Rays 4-6, broad, acute, often long acuminate, subequal, many revolute under the spore sac, rigid, thick. *Fleshy layer* more or less cracking and peeling off, army brown. *Exterior* more or less covered with dirt and debris. *Endoperidium* sessile, subsaccate, 2-2.5 cm. across, sayal brown to dingy white with long weathering. *Mouth* sometimes flaccid and nearly empty of spores, definite, seated in a definite area, concolorous or slightly darker than the endoperidium, of a different texture, often fimbriate, short conical. *Gleba* warm sepia to bister. *Columella* inevident to clavate. *Capillitium* dark brown, 3-7 μ thick. *Spores* globose, 4-6 μ in diameter. *Epispore* verrucose.

Habitat: In leaf debris under trees.

Distribution: ARIZONA, Flagstaff, July, '15, W. H. Long, 19 plants, 5520; Casa Creek, 9-4-38, C. O. G., 12 plants, 8268; Workman Creek area, Tonto Nat. For., D. J. Stouffer, 9-17-46, 16 plants, 11407; Eagle District, Crook Nat. For., 3-24-47, 2 plants, 11444; Mt. Graham, 5-8-47, 1 plant, no. 11459. NEW MEXICO, Eureka Lodge near Cuba, 9-7-31, W. H. Long, 1 plant, 8849; Cloudcroft, Aug. 1899, E. O. Wooten comm. W. A. Archer, 1 plant, Lloyd Myc. Coll. no. 16481; Organ Mts., 1907, 1 plant, Lloyd Myc. Coll. no. 21054. TEXAS, Denton, 1908, W. H. Long, 1 plant, 8856; Austin, 7-12-00, 3 plants,



FIGS. 21-24, $\times 1$. 21, *G. limbatus*, black European form, 2 plants; 22, *G. limbatus*, light colored American form, 3 plants; 23, *G. saccatus*, form MINOR, 4 plants; 24, *G. triplex*, 2 plants.

11202; Denton (Long no. 7471), 2 plants in Lloyd Myc. Coll. no. 52584 as *Geaster saccatus* var. *major*; (Long no. 7481), 8 plants in Lloyd Myc. Coll. no. 51284 as *Geaster saccatus*; Huntsville, J. W. Stiles, no. 3, 10 plants in Lloyd Myc. Coll. no. 51285 as *Geaster saccatus*.

21. *GEASTER SACCATUS* form *MAJOR* Lloyd Myc. Writ. 1: 111.
1902 (FIG. 25)

Sporophore hypogeous in leaf debris, becoming superficial and expanding at maturity, then 2.5–5 cm. across, button long beaked. *Exoperidium* explanate to revolute, brittle, usually splitting to or beyond the middle. *Rays* 6–8, narrow, subequal, acute with long acuminate points, not hygroscopic but rigid and very thin. *Fleshy layer* adnate, natal brown to raisin black, shining, thin, brittle, not rimose. *Exterior* practically free of dirt and debris, cinnamon, becoming light buff with age. *Base* plane or convex with or without an umbilical scar. *Endoperidium* sessile, papyraceous, subglobose, subsaccate, 1–1.5 cm. in diameter, fawn color fading to tilleul buff under weathering. *Mouth* large, usually strongly projecting before much weathering, seated in a definite depressed area, darker than endoperidium, hair brown to drab gray with age, often fimbriate. *Peristome* sorghum brown fading to wood brown, of different texture from the endoperidium, conical. *Gleba* mummy brown. *Columella* ovate, pointed. *Capillitium* hyaline to slightly smoky, walls thick, lumen small, 4–4.5 μ thick, walnut brown. *Spores* globose, strongly verrucose, subopaque, very dark brown, 4–5.2 μ in diameter.

Habitat: Gregarious in leaf debris under junipers.

Distribution: ARIZONA, Eagle District in Crook Nat. For., 3–24–47, D. J. Stouffer, 2 plants, 11444. NEW MEXICO, Jicarilla, Lincoln County, 7–18–41, D. J. Stouffer, 7 plants, 9543; 5–21–41, 5 plants, 9450; Ranger Tank near Corona, 5–12–41, 14 plants, 9329; Jicarilla, Lincoln County, 9–16–41, W. H. Long & D. J. Stouffer, 15 plants, 9757.

22. *GEASTER FORNICATUS* (Huds.) Fr. Syst. Myc. 3: 12. 1829
(FIG. 26)

Sporophore hypogeous in leaf debris with both universal and basal mycelium, becoming superficial and fornicate at maturity, 3–6 cm. across. *Exoperidium* revolute, bending strongly backward and downward, usually splitting beyond the middle. *Rays* 4–5, rarely more, subequal, tips attached to the fornicate mycelial layer, not hygroscopic, but thick and rigid. *Fleshy layer* dark brown to burnt umber, thick, more or less peeling off and leaving the surface of the fibrous layer naked and pecan brown. *Exterior* my-

celial layer stripped off from the fibrous layer when evagination occurs and remaining as a hollow cup attached to the tips of the revolute rays, often with a large hole in center where the basal mycelial mat was located. *Base* concave. *Endoperidium* pedicellate, stalk usually white when not weathered, subglobose to depressed-globose to urceolate, usually constricted at base into an apophysis, light drab to drab to cinnamon drab to often blackish brown, soft, finely velvety, 1–2 cm. across. *Mouth* indefinite without peristome but usually lighter in color than the endoperidium, conical to tubular apex which is often fibrillose to lacerate, sometimes white. *Gleba* seal brown. *Columella* inevident. *Capillitium* 3–5 μ thick, dark brown. *Spores* globose, 4–5 μ diameter. *Epispore* umber, finely verrucose.

Habitat: Gregarious in leaf debris under conifers and hardwoods.

Distribution: ARIZONA, Sabino Canyon area, 11–11–38, *W. H. Long*, 2 plants, 8265; 2–20–38, *W. H. Long & V. O. Sandberg*, 9 plants, 7681; 8 miles of Nogales, 11–10–38, *W. H. Long*, 13 nearly black plants, 8264. NEW MEXICO, 25 miles N.W. of Corona, 4–20–40, *W. H. Long & D. J. Stouffer*, 260 plants, 8748; Corona area, 9–15–41, *W. H. Long & D. J. Stouffer*, 56 plants, 9665; Sept. '40, *D. J. Stouffer*, 35 plants, 9122; Atkinson sandhill area, 25 miles west of Corona, 12–15–41, 13 plants, 9967; 4–21–42, *W. H. Long & D. J. Stouffer*, 30 plants, 10211; Corona area, *D. J. Stouffer*, 2–19–40, 7 plants, 8459; 20 miles N.W. of Corona, 1–2–41, *D. J. Stouffer*, 11 plants, 9246; Corona area, 4–17–42, *W. H. Long*, 2 plants, 10080; Willard in Parida Canyon, 11–29–41, *D. J. Stouffer*, 3 plants, 9941; Corona area, 7000 ft. elevation, 2–28–40, 82 plants, 8475; 4–21–40, *W. H. Long & D. J. Stouffer*, 21 plants, 8727; 34 plants, 8475; 4–20–40, 2 plants, 8697; Cougar Mt. area, 4–21–40, 5 plants, 8694. TEXAS, Austin, 1901, *W. H. Long*, 2 plants, 346; 3–2–03, *W. H. Long & A. M. Ferguson*, 1 plant, 11190; *W. H. Long*, 1 plant, 11438; and 2 black plants, 11188 (*Long* no. 346), in *Lloyd Myc. Coll.* no. 52482.

This species is very common and in great numbers in the vicinity of Corona, New Mexico, especially under *Juniperus monosperma*. Specimens are often found which have been pushed out of the debris by the alternate thawing and freezing of the soil, thereby making them easily visible to the collector where before they were very inconspicuous.

23. *GEASTER RUFESCENS* (Pers.) Fries, Syst. Myc. 3: 18. 1829
(FIG. 27)

Sporophore hypogeous in soil, becoming superficial and expanded at maturity, then 5–7 cm. across. *Exoperidium* explanate to revo-

lute to involute under the spore sac, very rigid but not hygroscopic, splitting to the middle or beyond. *Rays* 5-6, broad but acute, subequal, some involute at base of spore sac, rigid, thick. *Fleshy layer* thick, often porous and spongy, or cracked but not peeling off, Vandyke brown. *Exterior* more or less covered with dirt and debris, which often peels off under weathering leaving the rays naked beneath. *Endoperidium* very short stipitate, stipe thick with a broad apophysis, 2-2.5 cm. across, pecan brown to pinkish buff. *Mouth* indefinite, without a definite border, naked, concolorous or slightly darker. *Gleba* sepia. *Columella* globose. *Capillitium* 4-5 μ thick, sub-hyaline. *Spores* globose, 4-5 μ in diameter. *Epispore* slightly verruculose.

Habitat: In soil.

Distribution: NEW MEXICO, White Mts. near Ruidoso, 10-10-16, *W. A. Archer*, no. 14, 1 plant, Lloyd Myc. Coll. no. 30423. TEXAS, Houston, 1-1-17, *George L. Fisher*, no. 61, 2 plants, Lloyd Myc. Coll. no. 53493; Denton, 12-22-08, *W. H. Long*, 1 plant, 2108; Bryan, in Brazos County, 3-15-36, *Dr. Walter Ezekiel*, 2 plants, 8449.

24. *GEASTER FIMBRIATUS* Fr. Syst. Myc. 3: 16. 1829 (FIG. 28)

Sporophore hypogeous in leaf debris, becoming superficial and expanded at maturity, then 1.5-4 cm. across. *Exoperidium* revolute, flaccid, splitting to the middle. *Rays* 8-11, subequal, acute, not hygroscopic, tips turning under when completely expanded, leaving the base shallowly saccate. *Fleshy layer* adnate, often rimose or splitting, pecan brown to walnut brown. *Exterior* covered with leaf debris which often peels off at the tips, exposing the underside of the rays, then pale pinkish buff to cartridge buff. *Base* concave, not umbilicate. *Endoperidium* sessile, often with a white pruinose bloom, subglobose, more or less saccate, 10-22 mm. in diameter. *Mouth* indefinite, naked, usually slightly lighter in color, and shading into the surrounding tissue. *Gleba* sepia. *Columella* subglobose, rather prominent. *Capillitium* brown. *Spores* globose, dark brown, 3-4 μ diameter. *Epispore* minutely verrucose.

Habitat: Gregarious in leaf debris at high altitudes 6000-9000 feet, under pines and junipers.

Distribution: ARIZONA, Santa Catalina Mts. near Tucson, 1911, *W. H. Long*, 1 plant, 8855. NEW MEXICO, 1916, *W. H. Long*, 2 plants, 2657; Pecos Cienega Ranger Station, 9-30-14, 15 plants, 4159; Oct. '14, 8000 ft., 10 plants, 4502; Pecos, 1 plant, 9146; 1916, 2 plants, 5673; Indian Springs, Gallinas District, 9-15-40, *D. J. Stouffer*, 22 plants, 9549; Corona area, 9-15-40, 2

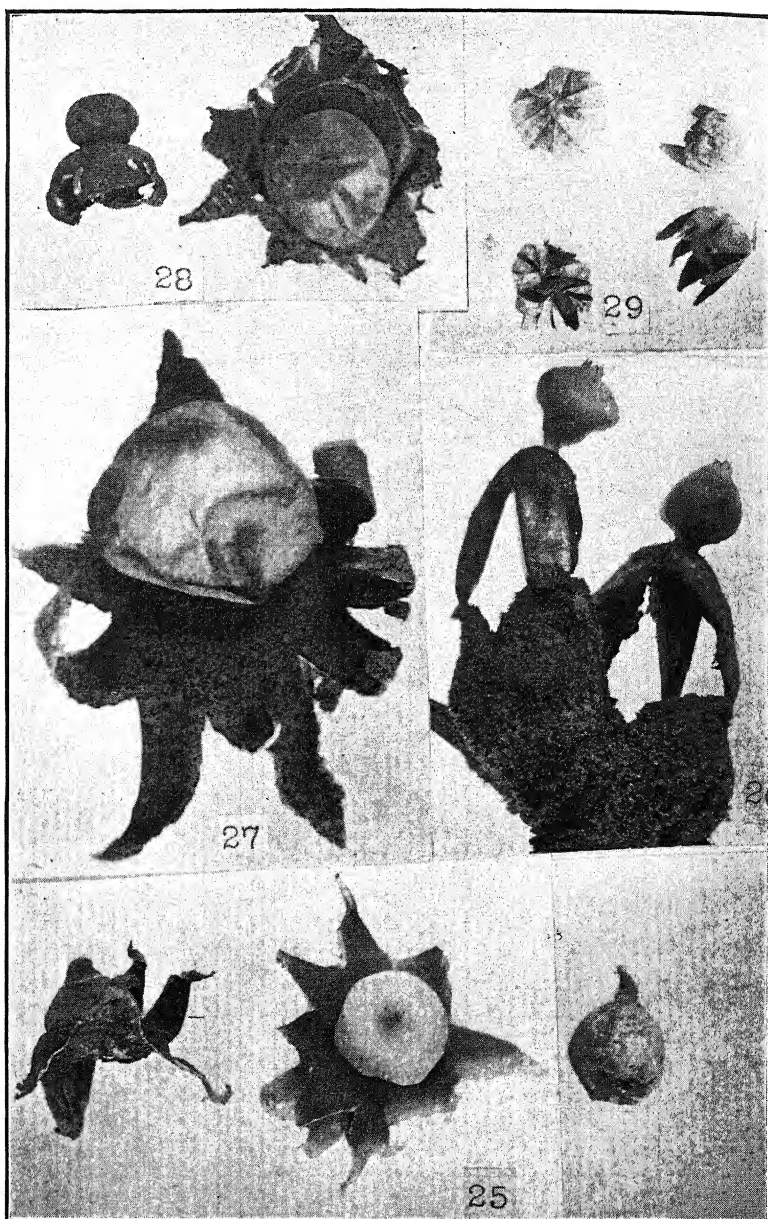
plants, 9163; April, '41, 1 plant, 9328; 4-4-40, 3 plants, 9272; Oct. '40, 1 plant, 9177; Sept. '40, 3 plants, 9127; 9-17-40, *W. H. Long & D. J. Stoutter*, 9 plants, 9876; 9-4-41, 2 plants, 9511; Ranger Tank, 5-12-41, *D. J. Stoutter*, 6 plants, 9313; 30 miles N.W. of Corona, 3-31-41, 1 plant, 9282; near Look-out Tower, 9000 ft., 2 plants, 9557; Indian Springs, 7700 ft., 9-5-40, *W. H. Long & D. J. Stoutter*, 245 plants, 9514. TEXAS, Austin, 7-17-00, *W. H. Long*, 1 plant, 11120; Bastrop, 7-9-42, *W. Z. M.*, 1 plant, 11439.

25. *GEASTER FLORIFORMIS* Vitt. Mon. Lyc. p. 23. 1842, p. 167.
1843 (FIG. 29)

Sporophore hypogeous with a universal mycelium, becoming superficial and expanded at maturity, then 1-2.5 cm. across. *Endoperidium* revolute when wet, strongly hygroscopic, splitting one half to two thirds the way to middle. Rays 6-12, very unequal, acute, rigid, hygroscopic and usually involute over the spore sac, covering it entirely, with the tips revolute when expanded. *Fleshy layer* adnate, continuous, not rimose, walnut brown to sepia. *Exterior* at first covered with a thin layer of mycelium and dirt, usually peeling off and leaving the lower surface of the fibrillose layer clean and free of dirt, then usually white, often with a white pruinose surface which shows up fine when the plants are wet, rarely pecan brown. *Base* convex to plane when rays are expanded, not umbilicate. *Endoperidium* sessile, membranous, 0.5-1 cm. diameter, subglobose, minutely furfuraceous to glabrate in age, not asperate, wood brown to army brown. *Mouth* indefinite, small, irregular, usually plane but rarely with a slight raised tube opening. *Gleba* umber to blackish brown. *Columella* ineventid. *Capillitium* subhyaline, about as thick as spores. *Spores* globose, 4-7 μ diameter. *Epispore* distinctly verrucose.

Habitat: Solitary or gregarious in small groups, in partial shade of piñons, pines and other desert vegetation.

Distribution: ARIZONA, Sabino Canyon area, 9-28-39, *W. H. Long*, 1 plant, 8775; 1-4-38, 1 plant, 8771; Flagstaff, 5-6-34, 15 plants, 8980; Nogales area, on Highway 87, 9-11-41, *W. H. Long & D. J. Stoutter*, 5 plants, 9690; Blue River area, Eagle Ranger District, Crook Nat. For., 3-24-47, 10 plants, 11446. NEW MEXICO, Corona area, 9-6-41, *W. H. Long*, 54 plants, 9567; 9-4-41, *W. H. Long & D. J. Stoutter*, 20 plants, 9502; Jornada Exp. Range, 9-8-41, 2 plants, 9716; White Sands National Monument, 9-13-41, 2 plants, 9755; 10 miles west of Deming, 9-12-41, 1 plant, 9700; Dulce, near Stone Lake, *Ledru Savage & R. L. Turney*, 6-25-34, 12 plants, 9023; San Ysidro, 7-19-41, *W. H. Long*, 3 plants, 9382; Eureka Lodge near Cuba, 8300 feet elevation, 11-1-37, *W. H. Long*, 2 plants, 9079. TEXAS, Denton, Pecan Creek, 9-17-07, *W. H. Long*, 20 plants, 2004; 10-10-07, 13 plants, 2028; Amarillo, 12-13-09, 28 plants, 2113; Denton, 9-17-07 (*Long* no. 2004),



FIGS. 25-29, $\times 1$. 25, *G. saccatus* form *major*, 3 plants; 26, *G. fornicatus*, 2 plants; 27, *G. rufescens*, 1 plant; 28, *G. fimbriatus*, 2 plants; 29, *G. floriformis*, 4 plants.

Lloyd Myc. Coll., 4 plants, 4726; 1-12-03 (Long no. 1791), 39 plants, Lloyd Myc. Coll., 4752; 10-22-06 (Long no. 1802a), 27 plants, Lloyd Myc. Coll. 4758; Dec. 1902 (Long no. 1780), 9 plants, Lloyd Myc. Coll. 4761; 6 plants, Lloyd Myc. Coll. 4773.

TERROSTELLA Long, Mycologia 37: 601-607. 1945

Geasteroides Long, Mycologia 9: 271. 1917.

Peridium double; *exoperidium* splitting into stellate, reflexed, persistent segments; *endoperidium* fragile, upper portion more or less deciduous, lower part persistent, consisting of a prominent sterile base; *mouth* single, *columella* and *capillitium* present.

TERROSTELLA TEXENSIS Long, Mycologia 37: 605-607. 1945
(FIG. 30)

Geasteroides texensis Long, Mycologia 9: 271. 1917.

Geasteropsis texensis (Long), Ed. Fischer, Engler & Prantl, Pf. Ed. 2, 7A; 75. 1933.

Sporophore hypogeous, becoming superficial and expanded at maturity, then 4-10 cm. across, usual size 6 cm. *Exoperidium* revolute, thick, coriaceous, subhygroscopic, splitting to about the middle. *Rays* 7-10, unequal, revolute, often with strongly involute acuminate tips 2-4 cm. long. *Exterior* with an outer layer of arachnoid mycelium and dirt that peels off as the plants age, leaving the exposed surface tiller buff to dingy white, often with faint longitudinal striae. *Fleshy layer* adnate, carob brown, fissured, cracked when dry, gradually wearing away. *Base* concave below and convex above. *Endoperidium* short pedicellate, subglobose, drab gray to light gray, 15-25 mm. broad by 18-20 mm. high, very fragile, apparently with a poorly defined mouth, upper portion slowly deciduous down to the sterile base, leaving it crowned with the subglobose columella and spores. *Sterile base* corky, compact, wood brown to fawn color, circular to oblong, circular ones 10-15 mm. across by 8-10 mm. tall, oblong ones 10 × 20 mm. to 25-27 mm. across by 10 mm. tall. *Pedicel* terete to strongly flattened, stout, subligneous, 2-4 mm. thick by 3-15 mm. wide by 2 mm. high. *Gleba* chestnut brown in very old plants, entirely disappearing and leaving only the sterile base seated on the pedicel (FIG. 30). *Columella* soft, weak, early deciduous. *Capillitium* wine color to light brown under the microscope, threads very long, distantly branched, 7-10 mm. thick, septate in the thicker portions, breaking up into segments 800-1000 μ long, walls smooth. *Spores* globose,

uniguttulate, 3-5 μ in diameter. *Epispore* brown, 1 mm. thick, faintly verruculose.

Habitat: Solitary or gregarious in small groups, around base of old rotting oak stumps (*Quercus stellata*) in open post-oak woods.

Distribution: TEXAS, Denton, 620 feet elevation, *W. H. Long*, 9-28-06, 4 plants, 1671; 10-8-07, 14 plants, 2011; 6 plants, 2034; Pecan Creek, 3 plants, 2035.

The distinguishing features of this species are its prominent corky sterile base and its fragile deciduous endoperidium. Specimens are deposited as follows: 6 plants from type material in Lloyd Myc. Coll. no. 8787 as *Trichaster texensis*, an herbarium name; 4 plants from type material are in the Herbarium of the University of California at Berkeley, no. 53941, under the name *Geasteroides texensis*; the remainder of the collections are in the Long Herbarium at Albuquerque, New Mexico.

ASTRAEUS Morg. Jour. Cin. Nat. Hist. Soc. 12: 19. 1889

Sporophore hypogeous with basal mycelium, becoming superficial at maturity. *Exoperidium* as in *Geaster*. *Endoperidium* membranous, dehiscent at apex, by a single mouth. *Columella* none. *Capillitium* long, much branched, originating from the inner surface of the peridium. *Spores* large, globose.

ASTRAEUS HYGROMETRICUS (Pers.) Morg. Jour. Cin. Soc. Nat. Hist. 12: 20. 1889 (FIG. 31)

Geastrum hygrometricum, Pers. Syn. Fung. p. 135. 1801.

Geastrum fibrillosum Schw. Naturf. Gesell. p. 113. 1822.

Geaster vulgaris Corda, Icones Fung. 5: 64. 1842.

Astraeus stellatus (Scop.) E. Fischer in E. & P. Aufl. 1 Teil 1, Abt. 1**:
341. 1900.

Geaster hygrometricus Pers. var. *giganteus* Lloyd. Myc. Writ. 1: 68. 1901.

Sporophore hypogeous, becoming superficial and expanded at maturity, then 1-8 cm. across. *Exoperidium* involute under or over the spore sac, rigid, splitting to the center of the base. *Rays* 7-20, usually very unequal, acute, strongly hygroscopic. *Fleshy layer* whitish when fresh, soon deciduous and leaving a rough rimose surface which may be mouse gray to benzo brown. *Exterior* free of dirt and debris, exposing the underside of the rays which are usually dingy white to cartridge buff. *Base* plane to convex, not umbilicate. *Endoperidium* globose to depressed-glo-

hose, sessile, 1-3 cm. in diameter, pitted or reticulate, whitish, becoming gray to brownish in age. *Mouth* an irregular torn aperture. *Gleba* sepia. *Columella* none. *Capillitium* much branched, threads hyaline, thinner than the spores. *Spores* globose, variable in size, 7-10 μ , average about 9 μ . *Epispore* verrucose.

Habitat: Solitary or gregarious in small groups in soil in open or partial shade areas, in sandy or clay soils.

Distribution: ARIZONA, Tucson, Santa Catalina Mts., 5-18-34, N. L. Noecker & R. C. Robeson, 10 plants, 9027; Pinal Mts., 8-1-34, 6 plants, 9016; between Miami and Superior, 9-11-32, O. F. Cook, 4 plants in Myc. Coll., Bureau of Plant Industry; 6 plants, 1922, C. R. Orcutt in Myc. Coll., Bureau of Plant Industry; Huachuca Peak, 6-14-34, 4 plants, 9028; Young, Tonto Nat. For., Aug. '34, 12 plants, 7969; Williams, 7-12-33, W. H. Long, 16 plants, 8885; Grand Canyon, Aug. '30, Dr. R. B. Street, 3 plants, 7888; Flagstaff, Fort Valley Experiment Station, 7-1-15, W. H. Long, 10 plants, 5407; 10-15-15, 4 plants, 5479; 10 miles from Flagstaff, 5-2-33, 10 plants, 8852; 7-1-33, 7 plants, 8978; June, '33, 6 plants, 7801; Bill Williams Mt., near Flagstaff, 6-21-30, J. F. Normand, 9 plants, 8866; Prescott, 2-16-34, V. O. Sandberg, 6 plants, 7616; 7-12-33, W. H. Long, 11 plants, 7768; 6 plants, 7825; 8 plants, 8850; 8-1-33, 6 plants, 8962; 1-3-33, 8 plants, 9058; Sitgreaves Nat. For., 11-15-45, D. J. Stouffer, 1 plant, 11083. NEW MEXICO, Corona area, 4-17-42, W. H. Long & D. J. Stouffer, 7 plants, 10060; State College, 1907, F. O. Wooten, comm. W. A. Archer, 1 plant, Lloyd Myc. Coll. no. 50121; 4-17-42, Red Cloud Picnic Grounds, 8300 ft., 36 plants, 10216; Oak Shinnery, 34 miles east of Roswell, on Highway 380, 4-19-42, 53 plants and 7 eggs, 10106; Santa Fe area, 7-19-36, J. N. O'Bryan, 2 plants, 5619; near old Alamos School area, 8200 ft., 5-10-33, W. H. Long, 10 plants, 8877; 7-12-41, 5 plants, 9406; Tejano Canyon, 7-30-16, 16 plants, 5655; 5-19-16, 6 plants, 5672; near Taos, 6-25-33, T. R. Moberg, 4 plants, 7823; Tres Ritas, Cienega Canyon, 10-19-14, W. H. Long, 6 plants, 4463; Silver City, 7-26-36, 14 plants, 8245; 7-7-33, 6 plants, 8982; 1911, 4 plants, 8868; Animas Mts., 7-26-34, N. L. Noecker & R. C. Carlson, 14 plants, 8785; Corona area, 9-15-41, W. H. Long & D. J. Stouffer, 6 plants 9753; Jicarilla, Lincoln County, 9-16-41, 5 plants, 9763. TEXAS, Denton, W. H. Long, 1907, 16 plants, 2054; Bastrop, 11-14-36, 1 plant, 11192; Valentine, 9-25-32, E. W., 2 plants, 11181; Austin, 3-2-1903, W. H. Long & A. M. Ferguson, 1 plant, 11121; San Antonio, June, '32, H. B. Parks, 5 plants, 11187; Austin, 7-1-01, W. H. Long, 2 plants, 11440; (his no. 1826), 19101, 4 plants, Lloyd Myc. Coll. no. 57090; Tyler, 1892, Wm. R. Harris, 7 plants in Myc. Coll., Bureau of Plant Industry.

Cunningham (1944) transferred this plant back to the genus, *Geaster* (*Geastrum*), with the following explanation (p. 178): "The treatment of this species by certain taxonomists well illustrates the pitfalls that lie in wait for those who worship at the shrine of ontogenetic classification. Morgan was the first to claim

that the plant which for nearly a century had been regarded as a typical *Geastrum*, differed sufficiently in several characters to warrant its being placed in a separate genus; to this he gave the name *Astraeus*, his reasons for its erection being that the species did not possess a regular hymenium or columella, the spores were larger than those of any other, and the capillitium showed certain differences from the other species.

"The only feature of those outlined in which the species differs from others of *Geastrum* is the somewhat primitive hymenium. In the developing plant the glebal cavities are separated by tramal plates to tenuous as to be overlooked by the uncritical worker. Each cavity is filled with basidia somewhat irregularly arranged in clusters (like those of *Scleroderma*) and not in the definite palisade of the species which have been studied. This difference disappears as maturity is reached, when the plants resemble closely the fructification of any other member of the genus. The taxonomist is then unable to indicate any point of difference by which '*Astraeus*' may be separated from *Geastrum*, which indicates that the name should be discarded.

"Morgan was content to retain the plant in the Lycoperdaceae; not so Fischer (1900), however, for without regard to the absurdity of such a treatment, he placed *Astraeus* in the Calostomataceae. And Kambly & Lee (1936) proceeded further and isolated it under the Astraceae of Martin!"

We are holding the genus *Astraeus* in deference to the general opinion of many recent taxonomists, but personally we incline to handling it as Cunningham has. We believe that after all the *mature* stage of a species, irrespective of any embryonic stages through which it might have passed and which have disappeared on maturity, should be the basis for its classification. The taxonomist can judge only by the characters found in the mature stage, now present and visible, where the plant should be placed.

MYRIOSTOMA Desv. Jour. de Bot. 2: 103. 1809

Sporophore hypogeous, becoming superficial at maturity. *Exoperidium* coriaceous, splitting in a stellate manner. *Endoperidium* membranous, opening by several to many mouths, columellae several, slender. *Capillitium* threads free, unbranched.

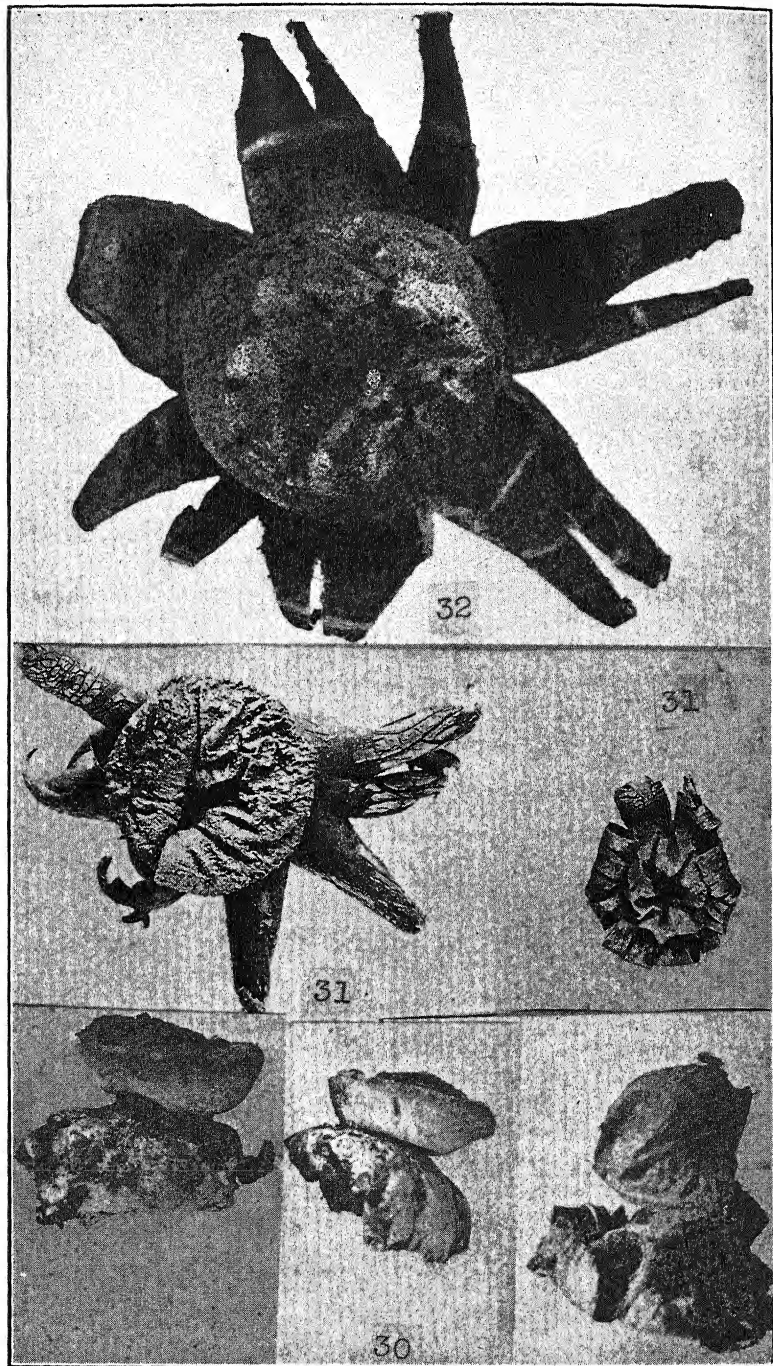
MYRIOSTOMA COLIFORME (Dicks.) Corda, Anleit., p. 204. 1842
(FIG. 32)

Bovistoides simplex Lloyd, Myc. Writ. 6: 883. 1919.

Sporophore hypogeous with a basal mycelial strand, often in leaf debris becoming superficial and expanded at maturity, then 2-12 cm. across. *Exoperidium* revolute, splitting from one fourth to nearly the middle. Rays 8-14, very unequal, tips often involute. *Fleshy layer* adnate when fresh, thick, often becoming deciduous in extreme old age, then cartridge buff to avellaneous, cinnamon to sayal brown when not weathered, becoming walnut brown under weathering, then thick and peeling. *Exterior* more or less with fine leaf debris or dirt but the dirt clinging to the concavity of the base. *Base* concave to vaulted, often showing a large umbilical scar where the mycelial strand was attached. *Endoperidium* on many having very short, inconspicuous pedicels, depressed-globose to subglobose, 1-5 cm. in diameter when not weathered, light cinnamon drab (silvery brown), cinnamon drab, to drab gray in age, minutely roughened with small subreticulate warts. *Mouths* several to many, ciliated, naked, apparently as many as the number of pedicels, mainly on upper half of spore sac. *Gleba* bone brown to burnt umber. *Columellae* several to many, usually inevident in the mature gleba, but apparent at base of spore sac. *Capillitium* long, slender, free, tapering, unbranched, 2-5 μ thick, with thick walls. *Spores* globose, 4-5 μ diameter. *Epispore* strongly warted.

Habitat: Gregarious in sandy soil, often in partial shade of trees.

Distribution: ARIZONA, Cardey, 4-1-36, V. O. Sandberg, 1 plant, 7722; Sabino Canyon area, W. H. Long, 11-10-38, 1 plant, 8309; 9-28-39, 4 plants, 8404; 2-20-34, 1 plant, 9621; W. H. Long & V. O. Sandberg, 9-22-30, 2 plants, 8020; 2-20-34, 2 plants, 7684; 11-10-39, W. H. Long, 1 plant, 8247; Eagle Creek District, Crook Nat. For., 3-31-47, D. J. Stouffer, 15 plants, 11451. NEW MEXICO, Corona area, 5-2-39, D. J. Stouffer, 1 plant, 8375; 2 plants, 8460; 4-3-40, 1 plant, 8695; Aug. '40, 3 eggs, 8759; Cougar Tank, 10-1-38, 6 plants, 8696; Corona area, 21 plants, 9167; Atkinson Ranch, 25 miles west of Corona, 12-15-41, 9 plants, 9968; 4-7-42, 9 plants, 10099; 4-15-42, 1 plant, 10238; 4-20-42, W. H. Long, 15 plants, 10118; 4-16-42, W. H. Long & D. J. Stouffer, 2 plants, 10054; Atkinson Ranch, 25 miles west of Corona, 4-21-42, 4 plants, 10213; Corona area, Jan. '40, D. J. Stouffer, 1 plant, 8441; 9-17-41, W. H. Long & D. J. Stouffer, 3 plants, 9782; 9-14-41, 32 plants, 9761; 9-17-41, 18 plants, 9736; 9-15-41, 15 plants, 9666; 28 plants, 9119; Nov. '39, D. J. Stouffer, 9 plants, 8442; 3-31-41, 30 miles N.W. of Corona, 12 plants, 9284; 4-21-40, W. H. Long & D. J. Stouffer, 31 plants, 8729; July, '40, D. J. Stouffer, 62 plants, 8710; Corona area, 7-28-40, 68 plants, 8711.



This species was very common and in large numbers in the Corona region as the above distribution records show. Long (1942) examined the type of *Bovistoides simplex* Lloyd and found that it was an old spore sac of a much weathered and depauperate specimen of the old and well known species, *Myriostoma coliforme*, which had become detached from the outer stellate exoperidium.

ACKNOWLEDGMENTS

We are indebted to Mr. John A. Stevenson for the loan of material and for many helpful suggestions and to Miss Edith K. Cash for editing the Latin diagnoses.

ALBUQUERQUE, NEW MEXICO
SAFFORD, ARIZONA

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FIGS. 30-32. 30, *Terrostella texensis*, 3 plants; 31, *Astraeus hygrometricus*, 3 plants; 32, *Myriostoma coliforme*, 1 plant; all $\times 1$.

TAXONOMIC NOTES ON LOUISIANA FUNGI—II. TREMELLALES

LINDSAY S. OLIVE

(WITH 3 FIGURES)

In this paper will be found descriptions of a number of jelly fungi collected in Louisiana during the past year. Since there seem to be few reports on this group of fungi in Louisiana, most of them will represent new records for the area. Twenty-seven species are included, four of which appear to be heretofore undescribed. It is expected that a considerably larger number of these interesting Heterobasidiomycetes will eventually be added to the present list.

EOCRONARTIUM MUSCICOLA (Fr.) Fitzp. *Phytopath.* 8: 212. 1918
(FIG. 2: 1-11).

This interesting fungus, which represents the type and only known species of the genus, is said to have a wide distribution. The elongate, slender fructifications, whitish at first and later yellowish tinged, were found growing on the sporophytes of a moss, probably a species of *Amblystegium*. They measure 4-7 mm. in length and are generally slightly swollen above. The texture is subfleshy. The hymenium covers almost the entire surface.

The fungus produces clavate, thin-walled probasidia from which the basidia arise. Fitzpatrick (1918) has shown that nuclear fusion occurs in the probasidium, followed by meiosis during the development of the basidium. The four-celled basidium is finally cut off at its base from the empty probasidium. The probasidium is never such a distinctly differentiated structure as is found in such fungi as the rusts. Furthermore, it is obvious that the probasidium is not of a sufficient size to produce a mature basidium by itself. Part of the contents of the basidium must be derived, during its formation, from the hypha bearing the probasidium.

The cells of the basidium produce conspicuous sterigmata of varying lengths. The basidiospores produced upon them are typically

falcate and measure $4.7-7 \times 21.8-31.3 \mu$. These spores frequently germinate by repetition.

Parasitic on sporophytes of a moss, probably *Amblystegium* sp., swampy woods near the campus, Baton Rouge, Louisiana; May 7, 1947; W. J. Dickson.

AURICULARIA AURICULARIS (S. F. Gray) Martin. Amer. Midl. Nat. 30: 81. 1943.

Numerous auriform fructifications of the "ear fungus" were found growing on rotten wood of a fallen deciduous tree. The collection was made in a woods near Baton Rouge, February 9, 1947.

HELICOGLOEA LAGERHEIMI Pat. Bull. Soc. Myc. Fr. 8: 121. 1892 (FIG. 1: 8-20).

This is one of the most widespread and also one of the most variable species in the genus. The genus is characterized by effuse, resupinate fructifications that are floccose and dry to soft-gelatinous in texture, and by the production of lateral, saccate probasidia that give rise to four-celled, transversely septate basidia. Two collections, varying considerably in certain microscopic characters, have been made in this area. In view of the variable nature of the species, the writer does not consider these to be of varietal significance.

The first specimen (FIG. 2: 8-14) was found growing on the underside of a dead oak limb lying on the ground. The fructifications are thin, not very extensive, and soft-gelatinous in texture. The surface is uneven and the color is grayish hyaline to gray. The probasidia, which are over 20μ long, and which are often characterized by having one or two constrictions, arise terminally on the hyphae, or sometimes in an intercalary position. They give rise to characteristic four-celled, rust-like basidia. The basidiospores in this collection are obovate and measure $6.1-7.6 \times 8.4-11.4 \mu$. This falls within the range, $4-13 \times 8-25 \mu$, given by Baker (1941), who has monographed the group.

The second collection (FIG. 1: 15-20) was obtained on the bases of old leaf petioles and fruiting stalks of the windmill palm.

The fructifications are similar in most respects to those of the first collection. Some of them have a somewhat pustular appearance and an olive-gray color. They dry to a thin, rough, olive-gray to dark layer. The hyphae, which are without clamp connections, measure $2.5\text{--}6\ \mu$ in diameter. The probasidia are terminal or rarely intercalary in origin and measure $9.6\text{--}12.2 \times 29.6\text{--}48.7\ \mu$. They germinate to produce basidia that measure $8.7\text{--}10.4 \times 66\text{--}125\ \mu$. The basidiospores are cylindrical, straight or slightly curved, and measure $8.7\text{--}12.2 \times 22.6\text{--}29.6\ \mu$. They frequently germinate by repetition. As can be seen by referring to the range given above, the basidiospores of this specimen reach a greater length than in any previously described collection. This further emphasizes the extreme variability of the species.

Both collections made on or near the campus, one on dead oak limb, March 2, 1947; the second on dead bases of petioles and fruiting stalks of the windmill palm, May 30, 1947.

***Helicogloea sebacinoidea* sp. nov. (FIG. 1: 1-7).**

Fructificationibus firmiter gelatinosis, sordide fusco-brunneis, latitudine ad centimetrum cubicum unum cum semisse patentibus, $2.5\text{--}4$ cm. longis, $150\text{--}765\ \mu$ crassis; superficie inaequali, verrucis parvis et nigris tecta. Hyphis typos duos habentibus, quorum alter hyphas angustas et brunneas $0.8\text{--}3\ \mu$ diametro, alter hyalinas hyphas latiores $3.8\text{--}12.2\ \mu$ diametro producit.

Probasidiis typice curvatis, per superficiem matricis gelatinosae hyphis maioribus effectis, origine et terminalibus et intercalariis, $10\text{--}14 \times 25\text{--}50\ \mu$ metientibus; basidiis transverse septatis, quattuor cellulas habentibus, rectis vel curvatis, $6.3\text{--}7.8 \times 70\text{--}122\ \mu$ metientibus; basidiosporis elongatis, rectis vel curvatis, $5.2\text{--}8.7 \times 10\text{--}14.5\ \mu$, uniseptatis frequenter existentibus. Haec species in ramo quercus demortuo crescit.

Fructifications up to 1.5 cm. broad and 2.5-4 cm. long, $150\text{--}765\ \mu$ thick, firmly gelatinous, color a dark sordid brown, not closely adherent to the substratum and separable from it, surface irregularly ridged and undulate, covered with small black warts consisting of conglomerate masses of dark hyphae, drying to a thin blackish brown crust and often curling back at the margins. Hyphae of two types, the narrow, brownish, much-branched hyphae most abundant, $0.8\text{--}3\ \mu$ in diameter, extending throughout the fructification but forming a dense surface layer above and below; large branched hyphae less numerous, $3.8\text{--}12.2\ \mu$ in diameter, extending to the upper surface of the fructification and producing the probasidia externally to the gelatinous matrix. Probasidia mostly curved, rarely straight, typically more swollen distally, both termi-

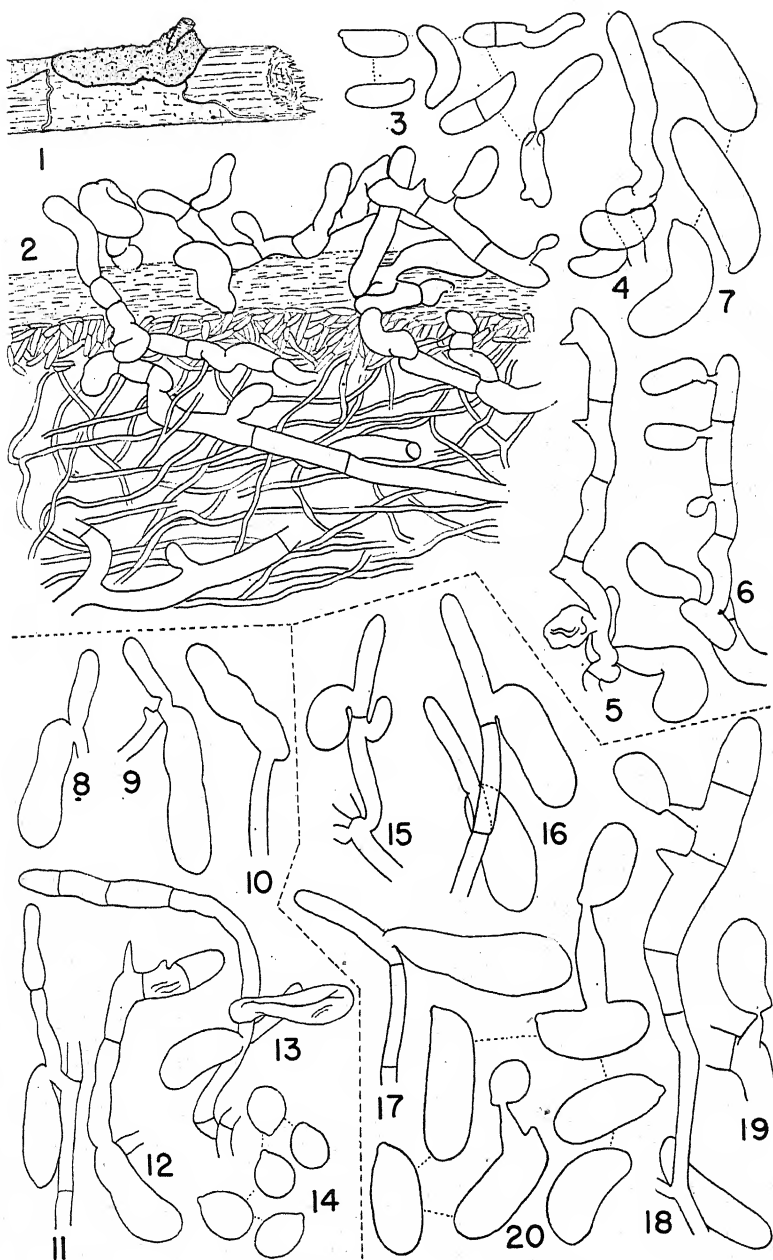


FIG. 1. Louisiana Tremellales.

nal and intercalary in origin, measuring $10-14 \times 25-50 \mu$, giving rise to transversely septate, four-celled basidia, basidia straight or curved, measuring $6.3-7.8 \times 70-122 \mu$, producing four basidiospores on relatively short sterigmata; basidiospores cylindrical, apiculate, straight to conspicuously curved, $5.2-8.7 \times 10-14.5 \mu$, germinating by repetition or becoming one-septate and germinating with germ tubes.

According to Baker's monograph (1936) of the genus *Helicogloea*, the fructifications of the various species vary in texture from mucous-gelatinous to floccose. In a more recent paper (1946), in which she gives a key to all the known species (eleven in number) of *Helicogloea*, she states: "The genus falls naturally into two lines depending upon the character of the fructification, which may be of the mucous-gelatinous ('tow-like') type, or the distinctly floccose (hypochnoid) type." The fungus described by the present writer, however, does not come within these limits, since its fructifications are decidedly firmly gelatinous. In other respects also the fungus differs from all previously described species in the genus. It now appears necessary to extend the concept of the genus to include forms with distinctly gelatinous fructifications.

The brown color of the fructifications is contributed primarily by the surface layer of narrow interwoven hyphae. The interior is only lightly tinted with brown. A most interesting feature of the fungus is the production of the probasidia just above the surface of the gelatinous matrix. As a result of this, both probasidia and basidia are entirely exposed throughout their development.

In general appearance and in texture the fungus is remarkably like some species of *Sebacina*. Even on drying, it resembles to a certain extent *S. adusta* Burt, which also frequently folds back at the margins when it dries. However, the dark warts over the surface would serve to distinguish it in the field from species of *Sebacina*.

Growing on a small corticate oak branch lying on the ground, in the vicinity of Shreveport, Louisiana; July 4, 1947.

TREMELLA LUTESCENS (Pers.) Fr. Syst. Myc. 2: 213. 1822.

The conspicuous orange-yellow, contorted fructifications of this jelly fungus were found to contain both the conidial and basidial

stages. The basidiospores measure $7.8-10.4 \times 12.2-15.7 \mu$. The fructifications were found on dead corticate limbs of frondose trees. Some grew on the dead but still attached branches of a locust. The collections were made in the vicinity of Baton Rouge; February and March, 1947.

Tremella rufobrunnea sp. nov. (FIG. 3: 4-18).

Fructificationibus gelatinosis, inaequaliter plicatis, colorem siennae ustae habentibus, ad centimetrum cubicum semis altis, $0.8-2.2$ cm. latis; superficie minutis punctis (vesiculis) tecta. Hymenio magnas vesiculas brunneas, $14.8-48.7 \mu$ diametro, conidia sphaerica usque ad formam praelongam, $2.7-4.6 \times 2.7-8.4 \mu$, atque basidia subglobosa usque ad formam globosam, verticaliter septata, $13.7-17.9 \mu$ diametro continente; basidiosporis ovalibus, $6.6-8 \times 9.1-12.5 \mu$. Haec species in frondoso ligno demortuo crescit.

Fructifications gelatinous, gyrose-folded, up to 0.5 cm. high and 0.8-2.2 cm. broad, color burnt sienna, surface minutely brown punctate when viewed with a lens (due to presence of globose vesicles protruding onto the surface singly or in groups); shrinking on drying and becoming dark brown or dark reddish brown. Hyphae with abundant and conspicuous clamp connections; hymenium containing conidia, basidia, and sterile vesicles; conidia produced with clamps at their bases, light yellowish-brown under the microscope, spherical to oblong or cylindrical, measuring $2.7-4.6 \times 2.7-8.4 \mu$; vesicles numerous, brown, inflated and somewhat thick-walled at maturity, protoplasmic contents eventually disappearing and wall often gelatinizing, many of them protruding onto the surface of the fructification, $14.8-48.7 \mu$ in diameter; basidia subglobose to globose, vertically septate, four-celled, the cells frequently showing a tendency to separate, $3.7-17.9 \mu$ in diameter; basidiospores mostly oval, apiculate, $6.6-8 \times 9.1-12.5 \mu$, germinating by repetition.

The fungus appears to be most closely related to *Tremella lutescens* (Pers.) Fr. (syn. *T. mesenterica* [S. F. Gray] Pers.), which has also been described as frequently containing inflated sterile cells similar to the vesicles of the new species. The two further resemble each other in their production of conidia and in the size of their basidia and basidiospores. On the other hand, the new fungus differs from *T. lutescens* in the decidedly different color of its fructifications, its less erect nature, the minutely brown-punctate surface, the color of the conidia and vesicles, and in the presence of a much larger number of inflated vesicles in the hymenium.

These characters seem to warrant separation of the two as distinct species.

Two collections of the fungus have been made. The first collection was found to be sterile. Its hymenial layer was filled with the sterile brown vesicles, but no basidia were observed. The specimen was found near Baton Rouge, but was discarded before the one described here was found.

Growing on dead corticate frondose limb, Avery Island, Louisiana; April 20, 1947.

TREMELLA FUCIFORMIS Berk. Hooker's Jour. Bot. 8: 277. 1856 (FIG. 2: 28-33).

Although the specimen described here differs in some respects from previous descriptions of *T. fuciformis*, there are many characters which indicate that it is that species. The fructifications occur singly or in patches up to 6×10.5 cm., while the individual fructifications measure 1.5-3.5 cm. in width and 0.7-1.2 cm. high. They vary from nearly translucent to opaque white in color, firmly gelatinous at first, becoming soft and mucilaginous, often sordid tinted, with age. The surface is much contorted or folded. The bases of the fructifications spread out into a yellow cartilaginous layer beneath the bark of limbs upon which the fungus is growing. The fructifications dry to a thin, yellowish, horny layer, or sometimes they merely shrink somewhat and retain some of their original form.

The hyphae are provided with clamp connections. Conidia are produced abundantly along with the basidia. These conidia appear in chains from the ends of some of the hyphae and are provided with clamp connections at their bases. They are subglobose to oval or elliptical, measure $5-9 \mu$ in diameter, and bud out smaller conidia of variable size. The basidia are four-celled, vertically or often obliquely septate, and measure $9.1-11.4 \times 11.4-16 \mu$. The basidiospores are broadly ovate and apiculate. They measure $4.6-7.4 \times 6.8-11.6 \mu$. They may germinate by repetition or bud out small conidia. The basidial cells and sterigmata also frequently bud out similar conidia.

This collection differs from previous descriptions of the fungus mainly on the basis of its larger basidia and basidiospores. Burt

(1921) states that the basidia measure $6-7.5 \times 7-10 \mu$ and the spores $4-4.5 \times 5.6 \mu$. Coker (1920) says that the spores are around 6.2μ in diameter. No one apparently has observed the presence of conidia in the hymenium. However, an examination of one of the specimens described by Coker revealed their presence. Our collection agrees well with Coker's in texture, color, size, and hymenial structure.

Growing on corticate oak limb, Colyell Bay, March 9, 1947.

TREMELLA MYCOPHAGA var. OBSCURA Olive. Mycologia 38: 540. 1946.

This interesting fungus was discovered originally as a parasite of *Dacrymyces*, namely *D. deliquescens*, *D. minor*, and *D. sp.* It has been previously reported only in Georgia and North Carolina. The present collection extends the host range to another genus of the Dacrymycetaceae. The fungus was found growing within the fructifications of *Dacryomitra stipitata*, where it produced both conidia and basidiospores in typical fashion. Collected near Varnedo, Louisiana; October 11, 1946.

EXIDIA SPICULOSA (S. F. Gray) Somm. Supp. Fl. Lapp. 307. 1826.

This and *Exidia recisa* are the most common jelly fungi in our area, as they seem to be throughout most of the country. The fungus is common on dead frondose wood all through the year. The writer has one collection of it on dead corticate pine branches. The fructifications were much more flattened out on the substratum than usual, but agreed in all other respects with the previous descriptions. The writer has found no previous record of this fungus on coniferous wood.

EXIDIA RECISA (S. F. Gray). Syst. Myc. 2: 223. 1822.

Like the foregoing species, this fungus is common throughout the year on dead frondose limbs.

EXIDIA NUCLEATA (Schw.) Burt. Ann. Mo. Bot. Gard. 8: 371. 1921.

The plant is easily recognized by its pinkish to vinaceous fructifications with white calcareous nodules embedded in them. A collection was made on a corticate oak limb, wooded area on edge of campus; June 22, 1947.

SEBACINA CINEREA Bres. Fungi Trid. 2: 99. 1892 (FIG. 3: 2 and 3).

The fructifications in our single collection of the fungus are thin, gray to grayish tan in color, and rather soft-waxy. The species is particularly characterized by the presence of golden colored, clavate to cylindrical gloecystidia in its fructifications. The basidiospores are oblong or broadly cylindrical and measure $4.6-6.3 \times 9.5-12.2 \mu$. These measurements fall within those given by McGuire (1941).

Growing on corticate and decorticate parts of an old oak limb, woods at edge of campus; June 22, 1947.

SEBACINA EYREI Wakef. Trans. Brit. Myc. Soc. 5: 126. 1915 (FIG. 3: 1).

Our collection of this species is characterized by its thin, waxy, grayish colored fructifications and its rather small cylindrical gloecystidia, which become yellowish in color. The basidiospores are globose and distinctly apiculate. They measure $4.2-5.3 \mu$ in diameter. The fungus corresponds very well with the description given by McGuire (1941).

Growing on a corticate frondose limb, Avery Island, Louisiana; April 20, 1947.

SEBACINA EPIGAEA (B. & B.) Bourd. & Galz. Hymen. Fr. 39. 1928.

In this collection, the fructifications are composed of small waxy-gelatinous, white to tan pustules. The basidiospores measure $7.5-9.6 \times 11.3-14.4 \mu$. One of the most characteristic features of this fungus is the tendency of its spores to become transformed into angular resting cells with somewhat thickened walls.

The fungus was collected in a single locality on a dead corticate oak limb and on the underside of some very old cow dung, wooded area near Baton Rouge; February 9, 1947.

SEBACINA ADUSTA Burt. Ann. Mo. Bot. Gard. 2: 764. 1915
(FIG. 1: 34-39).

The species has been frequently collected in this area. Its fructifications are effuse, waxy gelatinous to firmly gelatinous in texture, and dark brown or grayish brown in color. The surface is uneven and bumpy. The fructifications dry to thin, black, parchment-like layers which frequently split away from the hyphal layer next to the substratum and curl back around the margins.

The numerous paraphyses in the hymenium become encrusted with small brownish granules, which are probably primarily responsible for the brown color of the fungus. The paraphyses often gelatinize, after which the granules become scattered in the hymenium. Larger calcareous granules are also present in the fructifications. The basidiospores are curved-cylindric and measure $3.8-5.3 \times 9.9-16.7 \mu$.

This is probably the most common species of *Sebacina* in our area. It has previously been reported only in Idaho and British Columbia. Our collections agree rather well with McGuire's description, except that the fructifications are decidedly gelatinous in texture rather than fleshy.

Collected on dead frondose wood and on the dead bases of leaves and fruiting stalks of windmill palm. Common in this area throughout the year.

***Sebacina variseptata* sp. nov.** (FIG. 2: 12-27).

Fructificationibus effusis, exilibus, firmiter gelatinosis, fumo-griseis ad colorem olivaceo-griseum, superficie inaequali, parvis villis hypharum agglutinatatis tecta. Hyphis confibulas habentibus, paraphysisibus simplicibus vel ramosis, numerosa granula brunnea continentibus. Granula calcarea quoque in fructificationibus insunt. Probasidiis typice reticuliformibus, ad figuram pyri-formem aut ovoideam variantibus, basidiis quattuor cellulas habentibus, septis typice obliquis vel transversis, aliquando verticalibus, $7-10 \times 10.6-23 \mu$; basidiosporis allantoides, $3.8-5.3 \times 10.6-13.7 \mu$. Haec species in frondoso ramo demortuo collecta est.

Fructifications resupinate, thin, somewhat cartilaginous-gelatinous, smoke gray to olive gray, surface bumpy and covered with whitish tapering tufts of agglutinated hyphae; occurring in broken patches for an extent of 19 cm. along the limb, up to 5 cm. broad; drying to a thin tan to dark brown vernicose layer. Hyphae provided with numerous clamp connections, paraphyses simple or

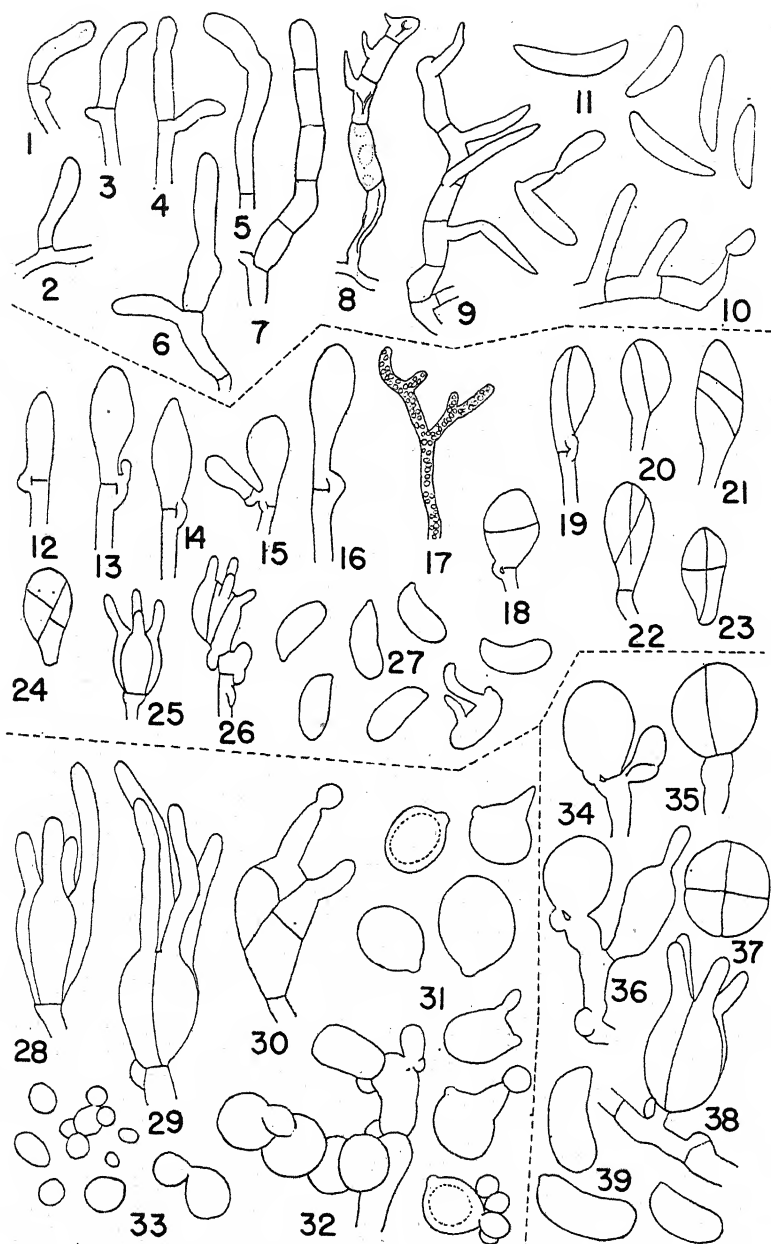


FIG. 2. Louisiana Tremellales.

branched, becoming filled with brownish granules, disintegrating and leaving the granules scattered through the hymenium; numerous calcareous granules also present in the fructification. Probasidia mostly clavate at first, typically swelling above and becoming racquet-shaped, or sometimes varying to pyriform or ovoid, commonly proliferating from clamp connections at their bases; basidia four-celled by means of septa which are typically oblique to transverse or sometimes vertical, measuring $7-10 \times 10.6-23 \mu$; basidiospores allantoid, $3.8-5.3 \times 10.6-13.7 \mu$, mostly germinating by means of germ tubes.

The closest related form in the genus *Sebacina* appears to be *S. adusta*, which the new fungus resembles in several respects. The two are very similar in texture and in the structure of the hymenium. Both have the characteristic small brown granules in the hymenium, as well as larger calcareous granules scattered through their fructifications. They are further alike in the shape and size of their basidiospores. On the other hand, the fungus described differs markedly from *S. adusta* in the great variability of its basidia and in its appearance when dry. In the present species, basidia with all septa vertical are rare, whereas basidia with all septa transverse to oblique are quite common. Moreover, these basidia are typically racquet-shaped, with a stalk-like base, and many of them reach a considerably greater length than do those of *S. adusta*. The latter species typically produces only ovate or obovate basidia in which the septa are all vertical. Also, the new species, when dry, does not split back around the margins as is characteristic of *S. adusta*.

In its basidial development, *S. variseptata* resembles to a great extent another tremellaceous fungus, *Patouillardinia cinerea* Bres., which has basidia characterized by transverse to oblique septa at right angles to each other. The two also have a somewhat similar texture, similar basidiospores, and calcareous granules scattered through their fructifications. The basidia of *P. cinerea*, however, are much longer, and there are other differences between the two. However, the fungus described here seems to represent an intermediate type which further links *Patouillardinia* phylogenetically with the genus *Sebacina*.

Collected on a corticate frondose limb, Avery Island, Louisiana; April 20, 1947.

SEBACINA PODLACHICA Bres. Ann. Myc. 1: 117. 1903.

Our collections for the most part fall well within the description given by McGuire (1941). An important point that does not seem to have been sufficiently emphasized is the frequent tendency of this and some other species of *Sebacina* to persist for some time in the form of small separate pustules, rather than as a continuous layer. In this way, they have much of the appearance of a *Stypella* or a small *Exidia*. In one collection obtained here, these little pustules were observed scattered over a piece of dead wood. They were connected only by a few strands of hyphae encrusted with tiny calcareous granules. The pustules were sporulating at this time. The resemblance to *Stypella* was outstanding. However, when the collection was placed in a moist chamber and in a cool environment for a few days, the pustules began to coalesce and assume the appearance of a typical *Sebacina* fructification. A somewhat similar phenomenon has been observed by the writer in *S. epigaea*.

The fungus appears to be fairly common in the state throughout the year on dead wood of frondose trees. Collected around Baton Rouge and Shreveport.

DACRYMYCES ABIETINUS var. *triseptata* var. nov. (FIG. 3: 19 and 20).

Fructificationibus pulvinatis, aurantinis ad colorem pallide flavum, firmiter gelatinosis, 2.5–10 mm. diametro, usque ad quinque centimetra cubica altis, frequenter coalescentibus. Basidiosporis maximam partem triseptatis, raro ad septem septa habentibus, $6.1-8.3 \times 12.2-22 \mu$, conidia globosa ad ovoida progemmantibus $2.1-4.5 \mu$ diametro. In pini ramis demortuis crescit.

Fructifications pulvinate, orange yellow to light yellow, fading with age, firmly gelatinous, later becoming soft, surface generally contorted, often cerebriform, individual fructifications measuring 2.5–10 mm. in diameter and up to 5 mm. high, some with small white radicating bases, coalescing into larger masses. Clamp connections absent, basidia typical, basidiospores measuring $6.1-8.3 \times 12.2-22 \mu$, becoming mostly three-septate with age, some up to seven-septate, budding out small globose to ovoid conidia, $2.1-4.5 \mu$ in diameter, or giving rise to germ tubes.

The fungus differs from previous descriptions of *D. abietinus*

(Pers.) Schroet. mainly on the basis of its larger fructifications and in the tendency of the great majority of its spores to become only three-septate, rather than seven-septate. Even after the specimen was left in the moist chamber for several weeks, by far the majority of its spores remained three-septate. Rarely, a few were observed with as many as seven septa. Also the basidiospores are, on the average, somewhat shorter than in *D. abietinus*. Previous descriptions of this species indicate that the basidiocarps vary from 0.5–4 mm. in diameter. A specimen collected in Georgia by the writer (1947) had basidiocarps that were 0.5–1.6 mm. in diameter. On the other hand, the new variety has fructifications that measure 2.5–10 mm. in diameter. The contorted appearance of these fructifications is also a distinctive feature. All of these differences are believed sufficiently important to distinguish the fungus as a new variety of *D. abietinus*. They do not seem of great enough significance to warrant separation of the fungus as a different species.

Growing on corticate and decorticate parts of some dead pine limbs lying on the ground, campus; March 14, 1947.

DACRYMYCES DELIQUESCENTS Duby. Bot. Gall. 729. 1829.

The species has been collected around Baton Rouge from fall to spring. It appears to be quite common here.

DACRYMYCES MINOR Peck. Ann. Rep. N. Y. State Mus. 30: 49. 1877.

The fungus was collected in a wooded area adjacent to the campus, where it was found growing on corticate frondose wood. May 14, 1947.

DACRYMYCES ELLISII Coker. Jour. Elisha Mitchell Sci. Soc. 35: 171. 1920.

Collected near the campus on a corticate frondose limb and on dead stems of a privet hedge; March and April, 1947.

DACRYMYCES PALMATUS (Schw.) Bres. Oesterr. Bot. Zeitschr. 54: 425. 1904.

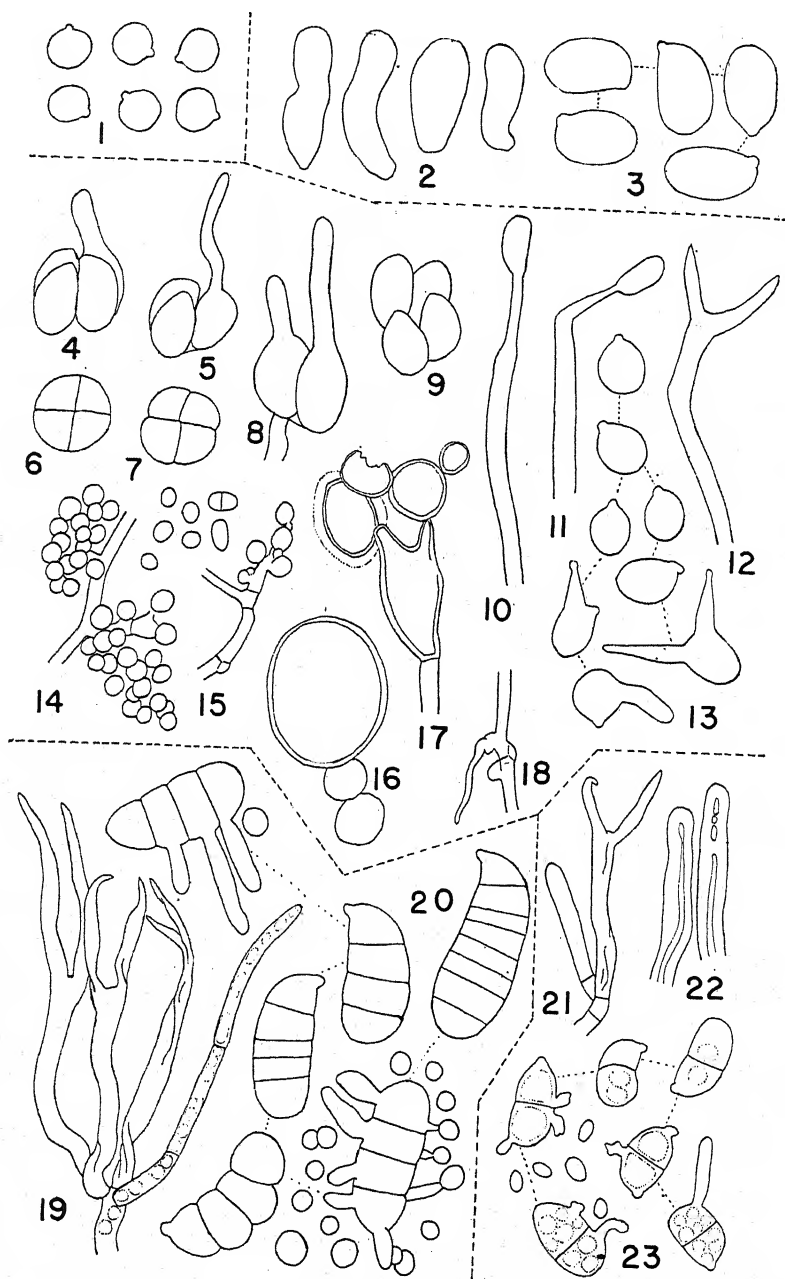


FIG. 3. Louisiana Tremellales.

Several large orange fructifications were found growing on a corticate pine log. The large basidiospores are seven-septate at maturity. Colyell Bay, Louisiana; March 11, 1947.

DACRYOMITRA STIPITATA (Peck) Burt. Ann. Mo. Bot. Gard.
8: 387. 1921 (FIG. 3: 21-23).

The small fructifications of this fungus arise singly or in groups from the substratum. They are elastic gelatinous and bright orange or orange-yellow in color. The fructification is usually provided with a small stalk and a pileus that is often somewhat convoluted or morcheloid and which is about 1-3 mm. broad. The fructifications are sometimes confluent near their bases, sometimes at the top. They measure 2.5-7 mm. in height.

The basidia are typical. The basidiospores are light yellow under the microscope. They become one-septate after being shed and measure $5.2-6.3 \times 9.9-12.6 \mu$ in one collection, $3.7-4.9 \times 10.1-12.8 \mu$ in another. The spores germinate to produce germ tubes or small globose to elliptical conidia.

This species seems to intergrade with *D. ceracea* (Coker) Brasf. with respect to most of its characters. They are certainly not distinguishable on the basis of color, texture, general structure, or basidiospore measurements. Brasfield (1938) considers *D. stipitata* and *D. ceracea* to be distinct species. In describing *D. ceracea*, he states: ". . . some basidiocarps may occur singly; when so, they are almost indistinguishable from *Dacryomitra stipitata* (Peck.) Burt; microscopically they are almost identical; however, the waxy consistency and the external appearance of *D. ceracea* serve to separate it readily from *D. stipitata*; the former is seemingly restricted to corticate oak wood." It is admitted in this statement that the two are sometimes macroscopically indistinguishable and that they are almost alike microscopically. The only other important criterion would appear to be the "waxy consistency." This latter concept, however, appears to be a mistake. Coker (1920) gave his fungus the specific name of *ceracea* because of the "deep wax-yellow" color of its fructifications and the "wax-colored" basidiospores. With regard to the texture of the basidiocarps Coker states that they are elastic gelatinous. In view of

these facts, the present writer can find no good reason for considering the two as distinct species. *Dacryomitra ceracea* (Coker) Brasf. is therefore considered here as being synonymous with *D. stipitata* (Peck) Burt.

Collected on dead corticate limbs of *Magnolia grandiflora* L., Varnedo, Louisiana; October 11, 1946, and on decorticate frondose wood, Baton Rouge; November 13, 1947.

GUEPINIA SPATHULARIA Fr. Elench. Fung. 2: 32. 1828.

Collected twice on old fallen trunks of deciduous trees, vicinity of Baton Rouge; May and November, 1947.

CALOCERA CORNEA (Fr.) Link. Handb. Gew. 3: 307. 1833.

Collected twice on unidentified dead wood near Baton Rouge; May and November, 1947.

ARRHYTIDIA INVOLUTA (Schw.) Coker. Jour. Elisha Mitchell Sci. Soc. 43: 237. 1928.

In our single collection of this species, the fructifications are thin (2 mm. or less in thickness) and have coalesced into patches 0.3–2.7 cm. in diameter. They are tough-gelatinous, closely appressed, and have a warty or gyrose surface and fimbriate margin. The color is orange-yellow when moist. On drying, the fungus shows reddish orange portions against a yellowish corticioid subiculum. No clamp connections have been observed.

The basidia are bifurcate and typical of the group; the basidiospores become one- to three-septate and measure $5.1\text{--}6.3 \times 12.6\text{--}17.1 \mu$.

Collected on dead corticate wood of cypress (*Taxodium*) Baton Rouge; October 21, 1946; W. J. Dickson.

GLOEOTULASNELLA PINICOLA (Bres.) Rogers. Ann. Myc. 31: 199. 1933.

The thin and effuse gelatinous fructifications of this member of the Tulasnellaceae were found growing over the bark of a dead oak limb and over the surface of an old resupinate leather fungus

on the limb. The species is one of the most common and also one of the most variable in the family. It has been reported in many parts of this country as well as in Europe. Collected near Baton Rouge; March 11, 1947.

The writer is grateful to Dr. P. G. Moorhead, Head of the Department of Classical Languages, for his preparation of the Latin diagnoses.

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EXPLANATION OF FIGURES

FIG. 1. *Helicogloea sebacinoides* (Nos. 1-7). 1, habitat sketch on dead wood; 2, section through fructification, showing large and small hyphae, also probasidia and basidia produced externally; 3, basidiospores, two-septate, one germinating by repetition; 4-6, stages in basidial development; 7, basidiospores much enlarged (No. 1, $\times 1$, nos. 2-6, $\times 460$, no. 7, $\times 1060$). *H. Lagerheimi* (Nos. 8-20). 1st collection: 8-13, probasidia and basidia; 14, basidiospores (Nos. 8-14, $\times 690$). 2nd collection: 15-19, stages in development of probasidia and basidia; 20, basidiospores (Nos. 15-20, $\times 570$).

FIG. 2. *Eocronartium muscicola* (Nos. 1-11). 1, 2, probasidia; 3-7, stages in development of basidia from probasidia; 8-10, germination of the basidia; 11, basidiospores (all $\times 460$). *Sebacina variseptata* (Nos. 12-27). 12-16, stages in development of the probasidia; 17, paraphysis with included granules; 18-26, stages in basidial development (all $\times 690$). *Tremella fuciformis* (Nos. 28-33). 28, 29, germinating basidia; 30, germinating basidium with conidium arising from apex of young sterigma; 31, basidiospores, some

budding out conidia; 32, hypha bearing conidia; 33, conidia, some budding in yeast-like manner (all $\times 690$). *Sebacina adusta* (Nos. 34-39). 34-38, stages in basidial development; 39, basidiospores (all $\times 690$).

FIG. 3. *Sebacina Eyreri*. 1, basidiospores ($\times 1060$). *Sebacina cinerea* (Nos. 2 and 3). 2, gloeocystidia; 3, basidiospores ($\times 1060$). *Tremella rufobrunnea* (Nos. 4-18). 4-9, basidia; 10-12, sterigmata; 13, basidiospores, some germinating by repetition; 14, 15, hyphae bearing conidia; 16, 17, vesicles; 18, hypha with clamp connections and appendage (all $\times 690$, except 16 and 17, $\times 460$). *Dacrymyces abietinus* var. *triseptata* (Nos. 19 and 20). 19, group of empty basidia and paraphysis; 20, basidiospores after septation, one germinating to produce microconidia, another producing germ tubes (all $\times 1060$). *Dacryomitra stipitata* (Nos. 21-23). 21, probasidium and empty basidium; 22, sterile, thick-walled cells from stalk of fructification; 23, basidiospores, some germinating to produce germ tubes or microconidia (all $\times 1060$).

AN EXAMINATION OF THE EXUDATE AND JUICE OF CERTAIN FUNGI FOUND IN THEIR NATIVE ENVIRONMENT

J. K. WILSON *

(WITH 1 FIGURE)

More than one hundred references exist in the literature relating to the exudative water given off by autotrophic and heterotrophic organisms. Certain ones referring to green plants go back about one hundred years whereas those referring to such organisms as fungi go back about seventy years. In these articles may be found data relating to the causes effecting such exudates as well as to their chemical composition. Since such exudates come from within the cell, any compound therein that is water soluble and can pass through the membrane of the cell may be found in the water. Little importance has been attached to this phenomenon but with our expanding knowledge of biotics, enzymes, phages, vitamins, viruses, and growth promoting substances such exudates should become of renewed interest. The early workers considered the exuded water to be about as pure as distilled water whereas later researchers found that the exudate from autotrophs may contain many substances although they may be present in relatively small amounts. The composition of water from certain autotrophic plants can be found in a paper by Wilson (1923).

Brefeld (1872) collected numerous drops from the surface of young sporangiophores of *Mucor Mucedo* and reported that the water had a weakly acid reaction. Wilson (1881) observed where such drops dried on the surface of the fungus—*Pilobolus crystallinus*—that they left radiating crystals, visible to the unaided eye. Lepeschkin in 1906, according to Buller (1934), analyzed the pressed juice and some of the exuded water from *Pilobolus lon-*

* Dr. Wilson passed away suddenly July 28. The proof of this article has been read by Prof. H. O. Buckman.

gipes. The pressed juice contained 4.1 per cent total solids whereas the exudate contained salts of the same nature but no organic substances were found in the latter. K, Na, Al_2O_3 , Fe_2O_3 , SO_3 , P_2O_5 , Cl, CO_2 and SiO_4 were recognized. The reaction of the drops was alkaline as determined by litmus paper and was due to K_2CO_3 . The exuding did not occur at 0°C . but was abundant at 35°C .

Knoll (1912) analyzed the drops from several species of fungi and reported that they contained a slime readily soluble in water and that as the drops dried up they gave an irregular wrinkled appearance.

Those workers who studied the exudate from fungi obtained their samples from organisms cultured under controlled conditions whereas the data presented in this paper comprise a study of the exudates and juices obtained from certain fungi growing under natural environments.

OCCURRENCE AND COLLECTING OF THE EXUDATE

The principal collections of the exudative water were obtained from *Polyporus dryadeus*. This fungus was growing on an oak stump and observations on the process of exudation were made over a period of sixty days. A photograph of the fruiting body with its exudate as seen at the most active period is presented in fig. 1. Because the stump was in the shade a mirror was used to reflect light so that a satisfactory exposure could be made. At times the water was so profuse that the stump and the soil were thoroughly drenched. The exudate began to appear in the morning after sunrise when the temperature of the air was rising and stopped late in the morning or early in the afternoon. This was observed to be true in five separate locations. If a drop became so large that it fell off, another small one would usually appear in a few minutes and within twenty to thirty minutes, sometimes a shorter period, it might be large enough to fall. About 10 ml. was obtained at the time the picture was taken. This was collected by touching the drops with a clean glass rod and by transferring that which was collected to a test tube. At times a pipette was used. A similar procedure was followed for obtaining the drops on *P. sulphureus* and

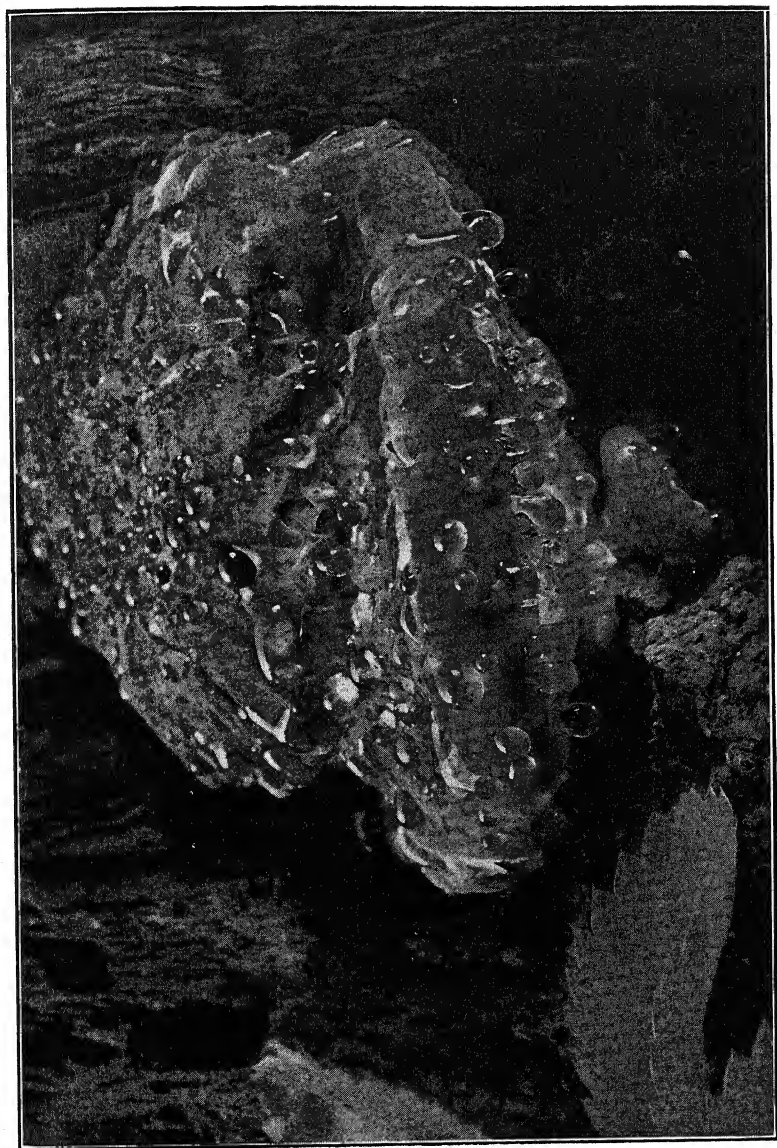


FIG. 1. Photograph of *Polyporus dryadens* growing on stump of an oak tree. August 4, 1947. About three-fourths natural size. The drops appear often at the same spot day after day.

on *Fistulina hepatica* although solution water was found only twice on the latter fungus.

CHEMICAL COMPOSITION OF THE EXUDATE

Known weights of the filtered exudate from *P. dryadeus* were evaporated to dryness in a weighed platinum dish on a steam bath and the total solids ascertained. The latter varied from time to time and ranged from 1.7 to 2.72 per cent. The solids were composed of organic and inorganic substances. Bacteriological examination revealed that the water contained from 6,000 to 32,100 bacteria and actinomyces in each milliliter. Despite the acidity of the exudate the bacterial growth increased more than 10,000 in each milliliter if the exudate was held for 3 days, but the population declined by the sixth day so that it was about the same as it was at the beginning. Comprising this population were three species of bacteria and one of actinomyces. Transfers of the exudate from the fungus to sterile agar slopes by means of a sterile loop were made also and the same types of organisms were present. Employing the alkaline methylene blue reduction test for carbohydrates it was evident that reducing substances were present. Both the Schiff reagent and the Orcinol test gave positive reactions for formaldehyde. If the Schiff reagent is specific and will detect one part of formaldehyde in a million then it can be said that there were present in the exudate at certain times 40 p.p.m. of formaldehyde. Tests were made to detect the presence of oxalic acid. To the acidic exudate calcium chloride was added, then followed by ammonia in water. Also in other tests hydrochloric acid and calcium acetate were added, followed by ammonia. No test was positive. Toward the middle of the day when exuding was ceasing certain droplets became smaller. This apparently concentrated the solids so that the residue became attractive to black ants. Such findings support those of Neger (1913) who wrote that the mycelia in ant hills secrete small drops of liquid that were readily taken up by the ants. Analyses of the exudate from *P. dryadeus* for certain inorganic constituents were made following the recommendations by Peech and English (1944). Recorded in p.p.m. there were K 500, Mg 110, Ca 25, P 1, Fe 0.5 and no Al or Mn.

ACIDITY OF THE EXUDATE

The acidity of the exudate from *P. dryadeus*, *P. sulphureus* and from *Fistulina hepatica* was determined within 15 minutes after the drops were collected. The exudate was placed in a Pyrex glass sample holder and a clean glass electrode of a Beckman pH meter inserted. Readings were obtained from many samples of each during July and August except those of *F. hepatica* which was available only twice. The pH findings follow:

	pH
<i>P. dryadeus</i>	2.60-2.85
<i>P. sulphureus</i>	3.57-3.80
<i>F. hepatica</i>	3.60-3.80

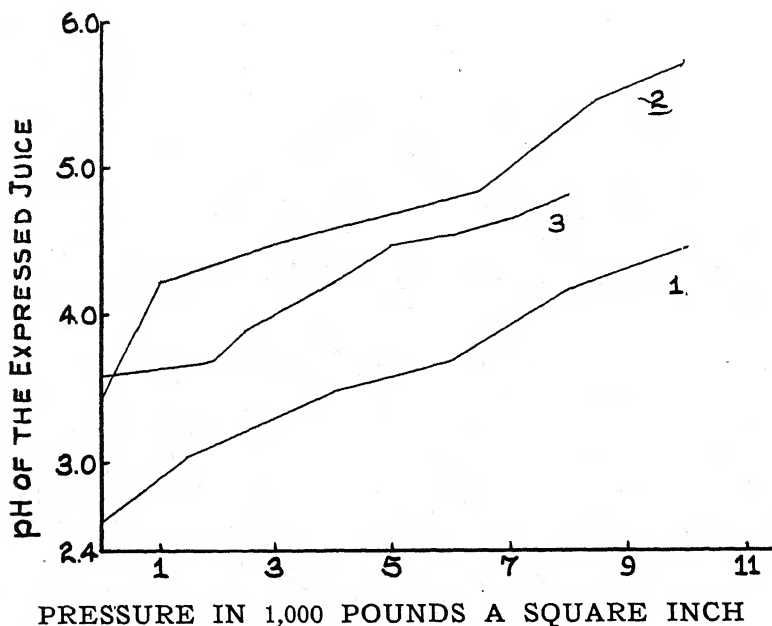
When collecting the exudate from *P. dryadeus* it was observed that the freshly exuded drops appeared perfectly clear whereas some of those that had fallen from one part of the growth to another and had remained there apparently for a considerable time were yellowish brown. Collections of the clear and of the colored exudate were obtained on the same day from the same growth. The clear liquid had a pH of 2.6 whereas that of the sample containing color was pH 4.4. The cause of this difference of color and acidity is unknown. It may be that certain parts of the growth yielded color that contaminated the exudate. Some of the clear exudate of pH 2.6 was boiled, then cooled, and its pH determined. It had not been changed and no color developed.

A sample of the exudate from *P. dryadeus*, the pH of which was 2.6, was diluted with distilled water to raise its pH value to 3.0. This required 13 parts of the water to 1 of the exudate. Also samples of the exudate with pH values of 2.6 were evaporated to dryness and the loss restored with distilled water. After thoroughly mixing, the pH values were again determined and were found to be 2.8.

ACIDITY OF THE EXPRESSED JUICE

The tissues of the three species of fungi collected from their native habitat were taken to the laboratory where the juice was expressed and the acidity of each determined. Cheese cloth was

folded around each sample—which in turn was placed in a cylinder of a Carver press. Gentle pressure was applied at first and a sample of the expressed juice was taken. As the pressure was increased other samples of the juice were taken also. The acidity of each was determined. The results are given in the accompanying graph, from which it is clear that the pH of the juice that was taken first was considerably more acid than that of the juice which came



GRAPH 1. Acidity of the expressed juice from fungal tissue obtained by gradational pressures. When the desired pressure was reached a sample of the juice was taken and the excess removed, then pressure was applied and another sample taken. This procedure was continued throughout. 1. *Polyporus dryadeus*, 2. *P. sulphureus*, 3. *Fistulina hepatica*.

out when the pressure was greater. The exudate from *P. dryadeus* was pH 2.6. The juice that came out by pressing to 1,000 pounds gave a higher pH value, and that which came out at a pressure between 8,000 and 10,000 had a pH value of 4.43. Increases in pH values were obtained also in comparable samples from *P. sulphureus* and from *F. hepatica* when similarly examined.

ACCOMPANYING OBSERVATIONS

It was observed during this study that many vinegar flies (*Drosophila cellaris*) were on and around the growth of *P. dryadeus*, yet the only sign that the fleshy growth was invaded was an occasional frothing. No larvae were seen at any one of the five locations where the fungi were found. The pressed juices from other fleshy fungi that were nearby had pH values near 4.8 and were almost destroyed by the larvae. In three of the five locations slugs (*Limax* sp.) were active and consumed large portions of the fleshy growth.

DISCUSSION

Exuding of water by certain fungi found in their natural environment is comparable in several particulars with that which occurs in green plants. Certain outstanding differences, however, are evident. The fungi start exuding in the morning when the temperature is rising and quit near midday, whereas the exuding by vascular plants starts when the temperature of the air in the evening is falling and may continue into the morning of the next day. According to numerous workers root-pressure is responsible for this phenomenon by plants. Since fungi have no roots this phenomenon in fungi cannot be a result of root-pressure. Either the factor causing exudation by the two organisms is the same or there is a different factor for each organism. In vascular plants activities continue while the temperature of the air is falling. Translocation of sugars and root-elongation are at a high rate at this time. Exuding by fungi is accelerated by rising temperature and thus increased metabolic activity should parallel this increase. Because the metabolic activities are parallel in both types of organisms—one bearing roots, the other bearing none—the part that root-pressure performs in this phenomenon can be questioned. This leads to the suggestion that the exuding of water by certain autotrophic and heterotrophic organisms is correlated with high metabolic activity. The flow of water with its solutes being probably in one direction only is in accord with the conclusions of Buller (1933) that the flow of protoplasm in the mycelium of *Fimetaria* is in the direction toward rapidly growing hyphae without any reversal.

The inorganic elements that were present in the exudate from *P. dryadeus* duplicate in many instances those that were found in the exudate of *P. longipes* (Buller 1934). Recorded in parts per million some of the elements were K 500, Mg 110, Ca 25, P. 1 but these represented only a small part of the total solids. The major portion apparently consisted of organic matter, although Buller (1934) says that none was found in the exudate of *P. longipes*, an allied species. This organic matter consisted of reducing substances as well as a transparent gelatinous residue, the composition of which was not studied. Apparently a part of this was cells of microscopic organisms for, like the exudate from green plants (Wilson 1923), the exudate from *P. dryadeus* contained many bacteria. The positive tests for formaldehyde were unexpected. One reagent indicated that this compound was comparatively abundant, equalling about forty parts in a million. This seems large in comparison with traces that may be found in the atmosphere. The observation that vinegar flies do not breed in the fleshy growth where the pH values were 2.6 but did in certain other fungal growths where the pH value was higher indicates that other fleshy fungi in which these flies do not breed may be strongly acid also.

SUMMARY

The exudates from three species of fleshy fungi growing in their natural environment were collected and examined. The total solids varied from 1.7 to 2.72 per cent. The pH value of each was low, being for *Polyporus dryadeus* 2.6. The water contained bacteria, actinomyces, reducing substances, a material giving the formaldehyde reaction and certain inorganic constituents. About 500 p.p.m. of potassium and about 1 p.p.m. of phosphorus were present. The exudate was highly buffered and contained a gelatinous material that was not studied.

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VARIATION IN FRUIT BODIES OF *CYATHUS STERCOREUS* PRO- DUCED IN CULTURE

HAROLD J. BRODIE

(WITH 3 FIGURES)

Lloyd (2) and other students of the Nidulariaceae or Bird's Nest Fungi have frequently remarked upon the variability of certain species, of which the well known coprophilous *Cyathus stercoreus* (Schw.) De Toni provides a good example. While this species is constant in its principal diagnostic features, the fungus cups show great diversity in different collections as to size, shape and color. Lloyd regarded the whole assemblage of described varieties of this *Cyathus* as representing but one variable species, and he reduced to synonymy with *C. stercoreus* about half a dozen very similar fungi that had been described as distinct species.

That at least part of the variability of this fungus has apparently a genetic basis is indicated by the writer's studies (1) of pure cultures. Various haploid mycelia were combined to produce a series of diploid mycelia, and, upon some of the latter, fruit bodies developed. The differences between the fruit bodies arising on the various strains were so striking that it seemed worth while to present a brief report on the matter.

Studies of *C. stercoreus* in this laboratory have thus far shown that the fungus is heterothallic and tetrapolar. Haploid mycelia exhibit marked differences from one another in color, texture and growth rate. When compatible haploids are paired, the diploid mycelia resulting show corresponding differences; there is every indication that a number of characteristics such as color, texture and growth rate are inherited. Thus, when white haploids are paired, the resulting diploid is white; when brown haploids are paired, the diploid is deep brown; when white haploids are paired with brown haploids, light brown diploids of various intensities of color are produced.

MATERIALS AND METHODS

Details concerning the source and manipulation of cultures of *C. stercoreus* have already been published by the writer (1), a brief restatement of which is pertinent to the present report. From a single peridiole of a fruit body of *Cyathus stercoreus* that appeared spontaneously on an old culture of horse dung in this laboratory, 60 single basidiospore cultures were obtained. Under ordinary conditions the basidiospores do not germinate readily, but it was found that heating them at 40° C. in distilled water for 48 hrs. induced about 60 per cent germination. Monospore mycelia are haploid and grow readily on Kauffman's agar, to which yeast extract and a trace of ferrous sulphate are added. When haploid mycelia are paired, the mating reactions show a regular tetrapolar pattern. Diploid mycelia, as they were obtained, were transferred to stock tubes of agar for further study. When it appeared that this organism might afford opportunity for genetic studies, it became desirable to induce and control the production of spore-bearing fruit bodies in culture. It had been observed that rudimentary fruit bodies occasionally develop on diploid mycelia grown on a variety of ordinary nutrient agar media. A search was then made for a medium which would give good mycelial growth and would enable the fungi to fruit. Ten variants of a basic formula were tested and the following medium was adopted merely because it gave maximum growth: agar 20 gm.; maltose 5 gm.; dextrose 2 gm.; glycerine 2 gm.; peptone 0.2 gm.; potassium acid phosphate (monobasic) 0.5 gm.; yeast extract 0.2 gm.; ferrous sulphate, trace; distilled water to make one liter of medium. Detailed study of the nutritional requirements of *C. stercoreus* has not been made, and it cannot as yet be stated that this medium satisfies completely the requirements of *C. stercoreus*. The medium has served, however, for the continuous and vigorous growth of over three hundred monosporous and diploid mycelia for over a year.

On this agar, a considerable number of diploid mycelia produce fruit bodies, which are generally small and abortive. By the addition of cellulose in the form of strips of filter paper, the medium was rendered capable of supporting the development of numerous fruit bodies, which were normal in their ability to produce viable

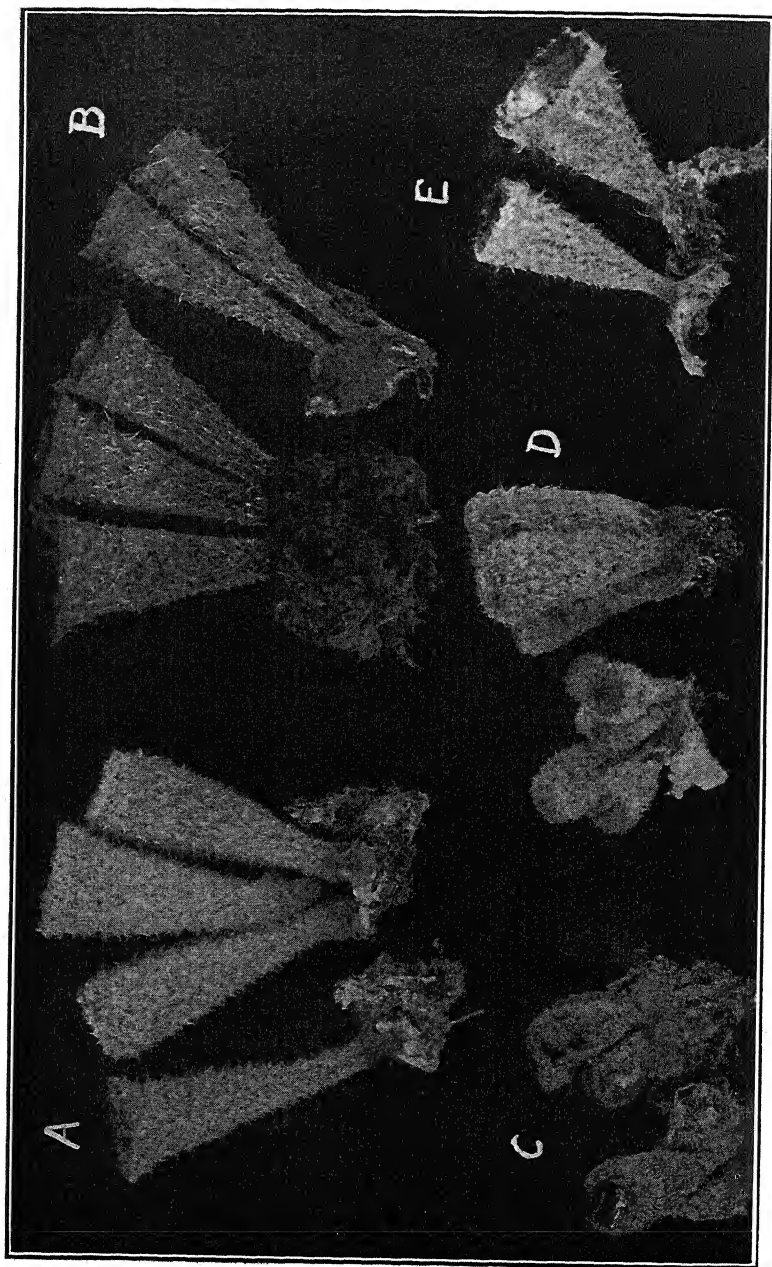


FIG. 1. Fruit bodies of *Cyathus stercoreus*.

basidiospores. Fruit bodies were also produced in abundance when diploid mycelia were transferred to jars of sterilized horse dung. On dung, the production of the fungus cups was more prolific but the fruit bodies did not differ appreciably from those produced on the agar medium. The fruit bodies shown in figures 1 and 2 were all obtained from diploid mycelia growing on aliquots of the agar medium. Although slight differences were observed between specimens produced in succession on any one culture, these differences were in no respect comparable to the differences between specimens arising from different cultures. Reference to figure 3 will make clear the genealogy of each of the cultures and fruit bodies described below. A series of haploid mycelia was obtained from a single peridiole of a single wild-type fruit body. These haploids were paired in all possible combinations and from those combinations which were fertile, diploid mycelia were obtained. Certain of the diploid mycelia produced fruit bodies in culture and these are described herewith.

No fruit bodies or even rudiments of them developed on any of the haploid mycelia even when these were transferred to the above mentioned medium.

TYPES OF FRUIT BODIES

Figures 1 and 2 show the mature fruit bodies that grew on seven different diploid mycelia of *C. stercoreus*. Each specimen (or group of specimens) is a representative sample of a series of fruit bodies. It will be seen that the various types show marked differences in size, shape and color. In some instances, the specimens are so unlike the fruit body from which the haploid cultures were obtained originally (wild type), that one would scarcely believe that they belong to the same species.

To facilitate comparison and reduce description, two tables are given showing the characteristics of the parent mycelia and of the fruit bodies. Table 1 sets out the appearance of the haploid parent mycelia and of diploid mycelia developed from them when the haploids were paired. These diploid mycelia produced the fruit bodies illustrated in the photographs. In Table 2, the characteristics of the fruit bodies are compared. A few comments about the different types may now be made.

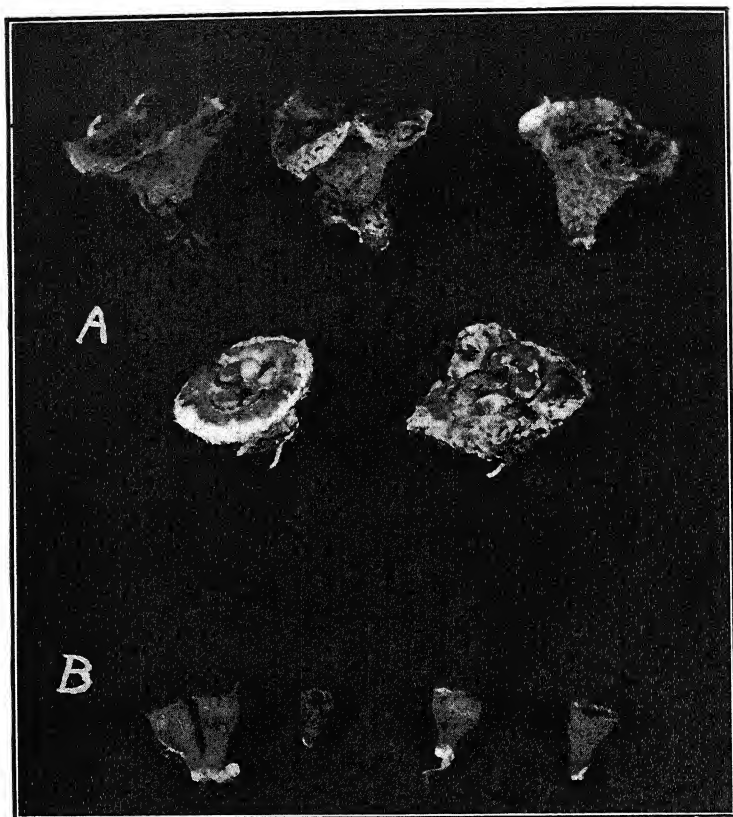


FIG. 2. Fruit bodies of *Cyathus stercoreus*.

1. *Color*.¹ The original wild type specimen from which all the cultures were derived and over two hundred fruit bodies produced by a diploid mycelium (No. N3.MM) obtained by culturing tissue taken from the base of the original specimen were all Cinnamon Brown according to Ridgway (4). Fruit bodies of the same color have also been obtained from a large number of the diploid progeny now in culture. These are not listed in Table 2 and will not be specially dealt with here.

¹Kodachrome transparencies have been made of mycelia and fruit bodies to provide a permanent record of color differences. Some idea of the differences in color between the various fruit bodies illustrated in figure 1 can be obtained from the photograph, since all the specimens were photographed together.

TABLE 1
CHARACTERISTICS OF SOME HAPLOID MYCELIA OF *Cyathus stercoreus* AND OF THE DIPLOID MYCELIA FROM WHICH FRUIT BODIES WERE OBTAINED

Haploid			Haploid			Diploid				
No.	Sex Genotype	Color (Ridgway)	Texture	No.	Sex Genotype	Color (Ridgway)	Texture	No.	Color (Ridgway)	Texture
53	aB	Pale ochraceous buff	Fluffy	29	Ab	Pale cinnamon	Fluffy	N3.MM*	Cinnamon to Sayal brown	Fluffy
53	aB	Pale ochraceous buff	Fluffy	2	Ab	Cartridge buff	Fluffy	53.29	Pinkish buff	Velvety
41B	ab	Mummy brown	Velvety	28S	AB	Pinkish cinnamon	Fluffy	53.2	Pale cartridge buff	Fluffy
33	aB	Pure white	Plaster-like	29	Ab	White to pinkish cinnamon	Fluffy	41B.28S	Sayal brown	Fluffy
33	aB	Pure white	Plaster-like	6	Ab	Ivory	Fluffy	33.29	White to very pale cinnamon	Fluffy
13	ab	Ivory	Fluffy	40	AB	Pale ochraceous	Fluffy	33.6	Cartridge buff	Velvety
33	aB	Pure white	Plaster-like	18	Ab	Ivory to cartridge buff	Fluffy	13.40	Pale clay color	Fluffy
								33.18	Cartridge buff	Velvety

* N3.MM

* N3.MM, tissue culture from original parent specimen.

TABLE 2
COMPARISON OF FRUIT BODIES DEVELOPED ON DIPLOID MYCELIA OF *Cyathus stercoreus*

Culture Number	Color of Cup (Ridgway)	Shape	Angle of Sides	Height, mm.	Diameter at Mouth, mm.	Dimensions of Peridioles, mm.	Spore Size in Microns	Outstanding Characteristics
N3.MM (FIG. 1B)	Cinnamon brown to Dresden brown; basal mycelial emplacement amber brown	Conical; mouth flaring slightly	75°	15-20	8-10	2 × 2.5	18-32 × 25-35	Cinnamon brown color, regular conical form, conspicuous brown basal mycelial emplacement.
53.29 (FIG. 2A)	Wood brown to buffy brown	Very broad funnel-shaped with strongly flaring sulcate mouth	60-70°	7-10	8-12	2 × 2.5	32-35 × 35-37	Grey brown color, extremely short cup with widely flaring mouth, peridioles not as black as in wild type.
53.2 (FIG. 2B)	Army brown to Natal brown	Regular conical, not flaring, minute	70°	4-6	3-4	1.5 × 1.75	32-35 × 32-38	Dark purplish brown, minute cups, small peridioles.
41B.28S (FIG. 1C)	Brussels brown with base amber brown	Conical, not flaring, malformed	80°	7-9	4-5	2 × 2.5	28-32 × 32-35	Deep brown color and irregular shape
33.29 (FIG. 1A)	Cartridge buff to cream buff with conspicuous russet base	Slender, flaring only at mouth	85°	15-20	6-8	1.75 × 2	22-25 × 32-40	Light grey brown with reddish base, slender form.
33.6 (FIG. 1D)	Cinnamon buff with cap ferruginous when young	Conical, not flaring at mouth	80°	10-12	5-6	1.75 × 2	28-32 × 32-36	Light buff color with reddish cap.
13.40 (FIG. 3)	Drab, with Brussels brown base	Broad conical	70°	10-12	8-10	1.75 × 2	25-35 × 32-35	Greyish brown, broad conical form and brown base.
33.18 (FIG. 1E)	Drab, without brown base	Broad conical	65°	10-12	7-10	1.5 × 1.75	25-35 × 32-36	Greyish brown, broad conical form, no brown at base, small peridioles

Some cultures produced fruit bodies darker in color than the wild type. For example, the cups developed on culture No. 41B. 28S (FIG. 1C) are Army Brown to Natal Brown, that is, considerably deeper in shade than the wild type.

Among the most remarkable of the series are those of light color, all of which developed on light colored diploid mycelia. The palest fruit bodies obtained to date grew from culture No. 33.29 (FIG. 1A). If one did not know beforehand that these fungi had been obtained by pairing certain haploids of *C. stercoreus*, one would be puzzled as to their species identity. It is interesting to note that Lloyd (2, p. 20) mentions specimens from India "of so light a color that I did not recognize them at first."

Variation in color is not confined to the cup as a whole. Certain parts of the fruit body may be specially colored in some cultures. All cups developed on culture No. 33.29 have a bright reddish brown base which shows (FIG. 1A) as dark shading at the base of each cup. Another interesting type is 33.6 in which the cup when young has a bright rust colored cap (FIG. 1D).

2. *Shape.* The original wild type specimen, and those produced by the tissue culture (N3.MM) from that specimen (FIG. 1B), were rather more elongate than are many wild specimens, especially those that grow on dung. The cups from several cultures are considerably more slender than the wild type, and have an attenuated base, as in culture No. 33.29 (FIG. 1A). Such slender stalked forms were referred by Tulasne (5) to the form *Lesueurii*. A broader type of cup was developed on several cultures, exemplified by No. 33.18 (FIG. 1E).

The most extreme deviation from the shape of the wild-type fruit body occurs in culture No. 53.29 (FIG. 2A). These extraordinary cups are broad and flat, some specimens being almost saucer-shaped.

The mouth of the fruit body of *C. stercoreus* is ordinarily not or only slightly flared. This condition is shown by the fruit bodies produced on culture No. 33.18 (FIG. 1E). However, extreme flaring develops in cups of culture No. 53.29 (FIG. 2A). In the latter specimens the cup is also strongly sulcate or furrowed, a character not at all typical of *C. stercoreus* but suggesting the flaring sulcate mouth of *C. vernicosus* (Bull.) DC.

3. *Size*. The majority of specimens developed in laboratory culture have been about as large as wild type specimens. As yet none has been obtained that is larger than the wild type.

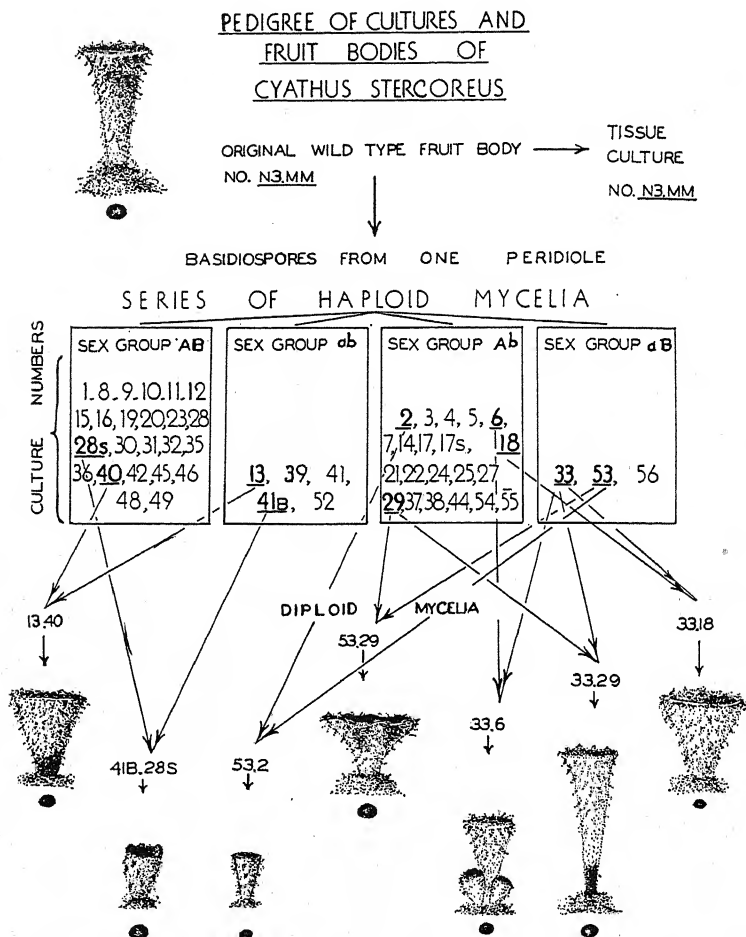


FIG. 3. Fruit bodies of *Cyathus stercoreus*.

One mycelium has produced minute cups. Specimens obtained from culture No. 53.2 (FIG. 2B) are dwarf and range from one fourth to one fifth the height of the wild type plant. Tulasne (5) referred small forms to the variety *minor*, concerning which Lloyd (2) remarks "there is no such thing as separating the various collections."

4. *Peridioles*. The peridioles in most cultures do not differ greatly in size from those of the wild type. Small peridioles, however, are characteristic of every specimen from cultures No. 53.2 and 33.18 (see TABLE 2 and FIG. 3).

Peridioles of *C. stercoreus* are typically black. One exception only has been noted in the cultures: the peridioles from cups of culture No. 53.29 are grey brown.

5. *Size of Basidiospores*. In the Nidulariaceae, basidiospores undergo enlargement after being dislodged from their basidia, a matter which was investigated by Martin (3). One therefore finds considerable variation in size among the spores of any one peridiole.

No conspicuous difference in size of the spores of the different strains was noted when fifty spores were measured for each culture. In some strains, the range in spore size is slightly greater than in others; but, in general, the mature spores are fairly uniform from strain to strain (TABLE 2). It is interesting to note that although the peridioles of 33.6 and of 13.40 are much smaller than normal, the spores within them are of average size.

GENERAL CONSIDERATIONS

That several distinct types of fruit bodies of *Cyathus stercoreus* can be produced in laboratory culture on diploid mycelia obtained from haploids, all originally derived from the spores of a single peridiole of one wild-type fruit body and then combined in various ways, is demonstrated by the foregoing account. Analysis of the genetic nature of these various types has not been completed, so that it is not known to what extent any self propagating lines may have been established.

One of the first questions that arises concerns the ability of the fruit bodies developed in pure culture to maintain themselves in nature. As yet we have no experimental evidence on this point, but it seems reasonable to infer that some of the forms of *C. stercoreus* described above, or other similar forms, exist in nature. Lloyd (2) and others who have studied these fungi have noted that the following types of fruit bodies may be collected in nature:

1. Short, unstalked forms usually growing on manure. These are considered by Lloyd as representing the type form of *C. stercoreus*.
2. Tall, slender forms with stalked cups. Lloyd assigns these to the form *Lesueurii*.
3. Small forms which Tulasne (5) called "var. *minor*."
4. Stalked slender forms with strong development of mycelium at the base of the plant forming a conspicuous brown ball. Lloyd refers these to the form *rufipes* and correctly points out that, as most species of *Cyathus* commonly develop a basal mycelial ball, this character is of doubtful value for species differentiation.
5. Extremely pale specimens such as those mentioned by Lloyd as coming from India.

Specimens that seem representative of all the groups listed above may be found among the different types described in this paper. In addition, the cultures have produced three types that do not appear to have been mentioned by collectors, *viz.*: 53.29 with its wide sulcate cups; 33.29 with its very pale cups, red brown at the base; and 33.6 with its cups provided with a reddish cap when young.

The stand taken by Lloyd seems justified, *viz.*, that *Cyathus stercoreus* is a variable species and that it is impracticable to separate from it as distinct forms (in some cases species) those specimens that are different in the shape of the cups, size of the cups and in some minor respects. On the other hand, practically all the specimens produced by the writer's cultures have the cup covered with shaggy hairs, have black peridioles devoid of tunica, and spores that are subglobose and between 30 and 40 μ in diameter. It would appear that these latter characters are therefore more reliable or less variable for the purpose of species definition in *C. stercoreus* than are the shape and size of the cups.

Relatively few of the basidiomycetous fungi that have been studied in pure culture have produced fruit bodies in culture. As a result, we have too little knowledge of the extent to which the differences between specimens of any one "species" collected in nature may represent variability inherent in the fungus and of the

extent to which the differences may be made the basis of a legitimate species concept. It is with this problem in mind that the present study of *C. stercoreus* is brought to the attention of mycologists.

SUMMARY

1. The coprophilous Bird's Nest Fungus *Cyathus stercoreus* has been induced to fruit in laboratory culture.

2. From a long series of haploid mycelia, all derived from spores of a single peridiole of one wild type specimen, a large number of diploid strains have been obtained that differ from one another in color, texture, growth rate and other characters according to the nature of the haploids from which they were produced.

3. Different strains of diploid mycelium fruiting in culture have produced fruit bodies of several different types, some so unlike the parental wild type as to bear little superficial resemblance to *C. stercoreus*.

4. The most distinctive types of fruit bodies illustrated and described are: a small deep brown abortive form; a tall slender, very pale form with a reddish brown base; a broad, flat, saucer-shaped form with sulcate cup; a minute or dwarf form; and a greyish form provided with a bright rust colored cap when young.

5. The characters of the species that have appeared least variable in culture are the hairy nature of the cup, the color of the peridioles and their lack of a tunica or outer covering, and the average size of mature basidiospores.

6. The possible connection between these culture types of fruit bodies and the various forms of *C. stercoreus* that have been described from nature is discussed.

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DESCRIPTIONS OF ILLUSTRATIONS

FIG. 1. Various types of fruit bodies of *Cyathus stercoreus* produced in culture: *B*, No. N3.MM (specimens developed on tissue culture from base of original wild type plant and identical with original parent specimen); *A*, *C*, *D*, *E*, various types produced on different diploid mycelia obtained by combining certain haploids, all themselves derived from spores of the single original parent specimen; *A*, No. 33.29; *C*, No. 41B.28S; *D*, No. 33.6; *E*, No. 33.18. $\times 2$.

FIG. 2. Two other types of fruit bodies of *Cyathus stercoreus* from same series as those shown in figure 1: *A*, No. 53.29; *B*, No. 53.2. $\times 2$.

FIG. 3. Diagram showing the origin of various types of fruit bodies of *Cyathus stercoreus* described and illustrated in this paper. From a single wild-type fruit body (No. N3.MM), many haploid mycelia were obtained. Pairings of certain of the haploids produced the diploid mycelia Nos. 13.40, 41B.28S, 53.2, 53.29, 33.6, 33.29 and 33.18. Each of these diploid mycelia gave rise to a distinct type of fruit body different from the original wild-type fruit body. Fruit bodies and the peridioles developed within them are represented natural size.

EMENDATIONS TO OUR PROPOSALS CONCERNING THE NOMENCLATURE OF THE GILL FUNGI

ROLF SINGER AND ALEXANDER H. SMITH

Since our proposals first appeared (*Mycologia* 38: 240-299, 1946), we have continued to check and recheck on the correctness of our data and the practical desirability of our proposals. This work has been helped by discussions with Dr. M. A. Donk who spent several months at the Farlow Herbarium, working on Basidiomycetes and comparing notes with the senior author on various aspects of nomenclature and systematics.

In spite of the fact that only five items have turned out to be, in one way or another, in need of emendation or correction, we believe that a speedy publication of the changes in the proposals is desirable even though they might be of no immediate nomenclatural importance or seem to be minor technicalities. They will, however, emend the set of facts on which we base our proposals, and will be of interest to those who, two years from now, must decide whether or not they will approve the proposals concerning the nomenclature of the gill fungi as outlined in our paper.

On p. 258, between XII and XIII, W. G. Smith's work, *Clavis Agaricinarum*, 1870, was omitted. In this, the genus *Lepista* (Fr.) W. G. Smith, l.c. p. 26, was first considered as a genus after it had been treated as a section of *Paxillus* by Fries in *Epicrisis* p. 315, 1838. The only possible, logical and practical type species, occurring both in Fries' and in Smith's definition of this taxonomic unit, is *Paxillus lepista* Fr. If this species, as we now propose, is considered as the *species typica* of *Lepista*, it turns out that *Rhodopaxillus* R. Maire becomes a synonym of *Lepista*, which has a solid priority. This is, of course, important only for those authors who separate this group from *Tricholoma* and *Clitocybe*. Aside from that, it makes our previous discussions on *Lepista* (see no. 73, p. 266, l.c. and no. 81, p. 269, l.c.) unnecessary. These should be considered as deleted.

On the same page (258), between XII and the above insertion,

we must insert the genus *Inocybe* (Fr.) Fr. which was given generic status by Fries himself in Monographia Hymenomycetum Sueciae 2: 346. 1863; not by Quélet ten years later as it may appear from our account (no. 72, p. 266, l.c.). Since Fries made this change in his comments on *Agaricus trechisporus* Berk., and since he expressly included rough spores as a character of the genus, it is necessary to propose a rough spored *Inocybe* as lectotype. Consequently we now propose *Agaricus trechisporus* Berkeley, Outl. Brit. Fungol. p. 156, 1869, as lectotype. Under these circumstances *Clypeus* and *Astrosporina* become synonyms of *Inocybe* and there is no valid generic name for the smooth spored species if they were to be considered generically distinct.

On p. 280, the type species of the genus *Geopetalum* Pat. is indicated as *G. petaloides*. We would now consider this choice as unfortunate since it has turned out that another one of the original species of this genus, *G. carbonarium* (A. & S.) Pat. (*Cantharellus carbonarius* A. & S. ex Fr.), is not congeneric with the *petaloides* group (whose legal generic name is *Hohenbuehelia*) and would remain without a generic name unless *Geopetalum* were revived in this sense. We therefore propose to replace *G. petaloides* (Bull. ex Fr.) Pat. by *Cantharellus carbonarius* A. & S. ex Fr. as the type species of the genus *Geopetalum*.

On p. 295 we proposed to consider the genus *Rhodophyllus* Quél. as nomen conservandum if used for a generic unit larger than the genus *Entoloma* (Fr.) Quél. Some mycologists seem to be opposed to this proposal because they believe it is out of line with the type concept. We believe it can be defended, but it now appears that the point is no longer applicable to the present case since the senior author (Singer, 1948, in press) has been able to prove the correctness of Burt's suggestion (1922) that the genus *Acurtis* Fries (1849) is the carpophoroid stage of *Rhodophyllus abortivus*. Fries placed the genus in the Clavariaceae. Thus the name *Acurtis* has definite priority over all the other names which might possibly be applied to *Rhodophyllus*. Those who insist on maintaining the artificial series of genera *Entoloma*, *Leptonia*, *Nolanea*, *Eccilia*, *Claudopus* and *Clitopilus* (not sensu R. Maire) would have to apply the name *Acurtis* to the species with decurrent gills and fleshy stipes which intergrade with some species of *Entoloma* whereas in the case of conservation of *Rhodophyllus*,

this latter genus would replace *Entoloma*. Those who now consider that all these genera are properly placed under one generic name would use *Acurtis* if they adhered to the rules. In either case the failure to conserve *Rhodophyllus* would introduce a name never used for any recognized group of gill fungi, and would make wholesale new combinations necessary. Our proposal to conserve the generic name *Rhodophyllus* Quél. conditionally is therefore herewith withdrawn, and we hereby propose, unconditionally, *Rhodophyllus* Quél. for conservation against *Acurtis* Fr., *Entoloma* (Fr.) Quél., *Leptonia* (Fr.) Quél., *Nolanea* (Fr.) Quél., *Eccilia* (Fr.) Quél., and *Claudopus* (Fr.) Gillet.

There is one possible source of disagreement which we wish to point out. Some might regard Article 65 of the Rules as applying in this case and discard *Acurtis* on that basis. The question is, is the carpophoroid stage of *Rhodophyllus abortivus* a monstrosity in the sense of the meaning of the Article or not? In our estimation Article 65 does not apply because this carpophoroid stage has been adequately demonstrated to be a characteristic (*i.e.*, normal) part of this organism. We do not deny that it is possible to disagree with this point of view on the basis of a different interpretation of the term "monstrosity." But this alternative interpretation would necessarily lead the argument to a discussion of the physiology of the organism involved. Since no experimental work has been done in this direction, the question would then be left open. Under these circumstances, the application of the name *Acurtis* is at least a threat to the continuity of nomenclature and, as such, should be eliminated by conserving *Rhodophyllus*.

On p. 290, the following two genera should be added: XXXV. Ex R. Kühner & R. Maire, *Etude de la réaction de la membrane sporique à l'iode*, Bull. Soc. Myc. Fr. 50 (1): 9-24. 1934.

199. ASPROPAXILLUS Kühn. & Mre., *l.c.* p. 13. *A. giganteus* (Fr.) Kühn. & Mre.

Status of generic name. Valid, but considered synonymous with *Clitocybe*, or *Paxillus* by some authors, by others considered synonymous with *Leucopaxillus*.

200. XEROMPHALINA Kühn. & Mre., *l.c.* p. 18. *X. campanella* (Batsch ex Fr.) Kühn. & Mre.

Status of generic name. Valid, accepted by many modern authors.

THE SWINGLE SPHACELOMA HAND LENS AND EARLY RECORDS OF THE PATH- OGENE OF CITRUS SCAB¹

ANNA E. JENKINS*

(WITH 1 FIGURE)

Fawcett (1, p. 2) has written that "the first extensive scientific investigations of Citrus diseases in the United States from the standpoint of cause and control were begun [in Florida] by W. T. Swingle and H. J. Webber in 1892." In the division of the work, as Dr. Swingle has told me, one of the citrus diseases he investigated was "scab or verrucosis."

An essential part of Swingle's technique in gaging the effectiveness of his experimental spraying for the control of scab was the use of a hand lens of unusually high magnification ($\times 40$). This had been made in New York according to his specifications. In publishing the results of this early experimental work he (6, p. 21-24) did not refer to his dependence upon this practical field tool. Quite by accident and only of late I learned of its existence. By Dr. Swingle's courtesy I was able to obtain a photograph of it for permanent record (FIG. 1, C). Early in my study of the scab pathogene I had the benefit of Swingle's manuscript drawings of the fungus as he examined it under the compound microscope. Certain of these are here published with his permission (FIG. 1, D). An ample specimen of scab on sour-orange leaves that Swingle col-

¹ This paper was published by title in the program of the Mycological Society of America, 11th Annual Meeting, Cleveland, Ohio, September 12-14, 1944. Since I could not attend, I found it opportune to discuss the subject at the Society's St. Louis meeting (March 27-31, 1946). This was in connection with my presentation relative to the Jenkins-Bitancourt *Myriangiales selecti exsiccati*, Fascicles 2-6 (4), of which fascicle 3 contains significant material of citrus scab (sour orange scab). In order that it may be available other than by the published title, the report, essentially in abstract, is published here.

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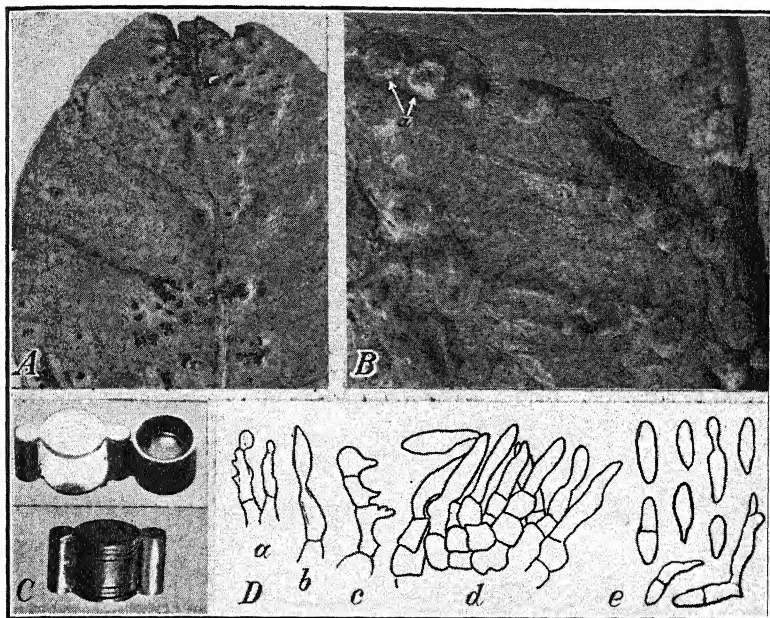


FIG. 1. *A* and *B*. Citrus scab on sour orange leaves showing the dusky conidial growth of the pathogene on their surfaces. (Part of *B* was published elsewhere (3, fig. 2, *C*) to show the light colored growth of a secondary *Fusarium* (*a*). Specimen from Gainesville, Florida, 1928. *A*, $\times 1$; *B*, $\times 6\frac{1}{2}$. *B*. Two views of the hand lens. $\times 1$. *C*. Swingle's manuscript drawings of the fungus showing individual conidiophores (*a*, *b*, *c*), clump of conidiophores arising from stromatic development beneath (*d*), and a group of detached conidia (*e*).

lected in Florida on April 12, 1892, appears as number 107 in the Jenkins-Bitancourt *Myriangiales selecti exsiccati* (4).

Swingle's early drawings and specimen of the fungus, as well as knowledge of the existence of the Swingle *Sphaceloma* hand lens, are essential to a satisfactory reconstruction of the taxonomic history of the organism. That eminent scientist's work with citrus scab belongs, of course, to the latter part of the "epoch-making period from 1885 to 1895" so termed by Galloway (2) in his graphic account of "Progress in the treatment of plant diseases in the United States." Dr. Swingle's continued researches in citrology have recently culminated in his "Botany of citrus and its wild relatives of the orange subfamily" (5).

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NOTES AND BRIEF ARTICLES

On ZYGODESMUS: *Zygodesmus* is one of that considerable number of genera that are, or ought to be, tagged with the condemnatory phrase "in the sense of Saccardo but not of the author." As Corda described it (Icones Fung. 1: 11. 1837) the genus was chiefly characterized by "flocci . . . geniculato-contracti, et dein per ramos vel geniculos transversaliter exsertis, conjugatis." The clamp-connections thus described (perhaps for the first time) and illustrated (Pl. 2, fig. 164, 165) gave the genus its name (Ζύγωω, join, + Δέσµα, link). The spores, "simplices, . . . pellucidae," were scarcely determinative. The two species originally assigned to the genus, *Z. Hypochnoides* and *Z. ochraceus*, are more likely to have been species of *Corticium* than anything else; but since the spore-bearing organs are not described, the species might possibly have been clamp-bearing Moniliaceae like those reported by Linder (Lloydia 5: 165-207. 1942) in *Oidium*. In either case, there is insufficient published detail to permit even the tentative identification of any known fungi with Corda's descriptions, and the type specimens are missing from Corda's herbarium in Prague (Pilát, Mus. Nat. Prague Acta 1B: 139-170. 1938). Both specific names are therefore *nomina dubia*, to be rejected under Art. 63 of the Rules. Since one or the other of these species must be the type of the genus, *Zygodesmus* is likewise a *nomen dubium*. We know only that it was published for fungi floccose, clamp-bearing, and hyaline-spored.

Three years after the publication of his genus Corda added to it a third species, *Z. fuscus* (Icones Fung. 4: 26. Pl. 6, fig. 81. 1840). This fungus had little in common with the earlier species, or with the generic description, beyond the bare possession of hyphae and spores. The hyphae were fuscous and without clamps, and the spores were spiny and fuscous. Just as the earlier species, if imperfect, as described, should be Moniliaceae, so *Z. fuscus* should belong to the Dematiaceae. It is this alien species which Saccardo, disregarding the clear implications of the generic name,

the generic description, and the original species, seems to have adopted as the typical one (Syll. Fung. 4: 283-288. 1886). At any rate, this is the one which he chose for the first place and most careful treatment in his group (? subgenus) *Euzygodesmus*; the original species are listed towards the end of a third group. Other authors have followed this lead, and Clements & Shear (Gen. Fung. 395. 1931) have formally designated *Z. fuscus* as the type of the genus.

Through the kindness of Dr. A. Pilát of the National Museum, Prague, the type specimen of *Z. fuscus* was borrowed for study. As could have been predicted, it proved to be a *Tomentella*, *T. biennis* (Fr.) A. M. Rogers.¹ The specimen is poor, lacking the discoloured hymenium and subhymenium so well described by Bourdot & Galzin (Hym. France 486 [1928]), and retaining only the fuscous-brown subicular hyphae and the spores; but from their apiculi the spores must be basidiospores, and the fungus not only can be traced in Bourdot's key but agrees in all respects with more perfect material of *T. biennis*.

Zygodesmus then presents one more case where "general usage . . . instead of priority of publication" (Clements & Shear, Gen. Fung. 15. 1931), if adopted as the basis for designation of types, would make a bad situation considerably worse. Not only is *Z. fuscus* not eligible for inclusion in the genus as described by Corda, but its adoption as type would necessitate that the name *Zygodesmus* Corda 1837 should supersede *Tomentella* Pat. 1887. As here shown, however, that is not only unnecessary but impossible.

What then are the fungi which authors since Corda have assigned to *Zygodesmus*? A dozen or more belong in *Tomentella*; at least five are members of *Pellicularia*; others belong to *Peniophora* and *Coniophora*. The genus is a mine of interesting resupinate Basidiomycetes. Since Linder has found that three species belong to *Oidium*, it is still possible that fungi will be discovered in *Zygodesmus* sensu auctt. which can properly be included within the Dematiaceae. But neither *Zygodesmus* Corda

¹ *Thelephora biennis* Fr., Syst. Mycol. 1: 449. 1821; *Tomentella phylacteris* [Bull.] Bourd. & Galz. apud Bourd. & Maire, Soc. Mycol. France Bul. 36: 81. 1920; *Tomentella biennis* (Fr.) A. M. Rogers comb. nov.

nor *Zygodesmus* sensu Saccardo belongs there.—DONALD P. ROGERS, NEW YORK BOTANICAL GARDEN.

RUSSULA TRICOLOR Murr.

Russula tricolor Murr. Lloydia 8: 272. 1945

Dr. Rolf Singer informs me that Roger Heim used this name before I did, so I propose *Russula patriotica* comb. nov. for my species.—W. A. MURRILL

PROPOSALS FOR THE AMENDMENT OF ART. 64 OF THE INTERNATIONAL RULES OF BOTANICAL NOMENCLATURE

Art. 64. A name of a taxonomic group must be rejected if the characters of that group were derived from two or more entirely discordant elements, especially if those elements were erroneously supposed to form part of the same individual. A list of names to be abandoned for this reason (*nomina confusa*) will form Appendix V.

Examples: The characters of the genus *Schrebera* L. (*Sp. Pl.* ed. 2, 1662: 1763, *Gen. Pl.* ed. 6, 124: 1764), were derived from the two genera *Cuscuta* and *Myrica* (parasite and host) (see Retz. *Obs.* VI, 15: 1791). The characters of the genus *Actinotinus* Oliv. (in Hook. *Ic. Pl. t.* 1740: 1888) were derived from the two genera *Viburnum* and *Aesculus*, owing to the inflorescence of a *Viburnum* having been inserted into the terminal bud of an *Aesculus* by a native Chinese collector. The names *Schrebera* and *Actinotinus* must therefore be abandoned.

Proposal 1: for "especially" substitute "and."

Argument: Although the wording of the Rule indicates that it may be applied to names of groups whose characters were derived from discordant elements *not* supposed to form part of the same individual, its only useful application is to names based on discordant elements *supposed* to form part of the same individual; and its application to names of the former sort must result in the discarding of very large numbers of well established names, and must constitute an invitation to extensive abuse.

The Rule first appeared as a proposal published without elaboration or explanation in the form: "Every one should refuse to admit

a name . . . when the group which it designates embraces elements altogether incoherent . . ." (Burnat, E., & Durand, T. Propositions de changements aux lois de la nomenclature botanique de 1867, p. 11 (Art. 53). 1903), and became Art. 51 (4) of the 1905 Rules. Such a phrasing being evidently ambiguous, an expanded statement was proposed by certain British botanists (International Botanical Congress, Cambridge (England), 1930. Nomenclature proposals by British botanists, p. 28 (Art. 69). 1929) and was incorporated unaltered into the Rules by action of the Cambridge congress. There is no record of interpretative discussion at either congress. It is, however, significant that although the British committee pointed out (*l.c.*, p. 43) that the generic name *Crinodendron* had been rejected by Sprague (under an earlier Rule) because it had "been shown to be based on a mixture of at least two species belonging to different families," and nevertheless was subsequently retained by Schneider, no attempt was made to support the opinion of Sprague by listing the case of *Crinodendron* among the Examples. On the contrary, these relate exclusively (*l.c.*, p. 28) to cases where the "discordant . . . elements were erroneously supposed to form part of the same individual." This creates a strong presumption that it is only to such cases that the Rule ought to be applied.

Actually, there is no need for any broader application of the Rule. Its text was written, just as the case of *Crinodendron* was judged, before the type concept had become a part of the Rules. The principle that is now applicable, and now generally applied, is that when two or more "incoherent" or "discordant" specimens form the basis for a specific name, or two or more "incoherent" or "discordant" elements (such as species) form the basis for a name of a higher rank, the name is "permanently attached" to "that constituent element of a group" (Art. 18) which was designated, or is selected, as the type. With the type concept forming a part of the Rules, there is then only one class of cases where a Rule like Art. 64 is needed—the cases where the type itself consists of "discordant elements"—*i.e.*, where those "elements were erroneously supposed to form part of the same individual." Such cases are not infrequent among fungi, where careful microscopic examination may be needed to disentangle the mixed individuals, and, as shown by

the Examples earlier referred to, they occur occasionally among vascular plants.

The literal application of the Rule as it stands would mean that every specific name published with a description drawn up from representatives of two species (or two subspecific categories), every genus drawn up for members of different genera, and so on, should by its operation be discarded. Since the species and genera of older botanists are often held by more recent ones to embrace two or more of such "elements" (*Agaricus* Fries, for example, including species now often assigned to different families, *Clavaria* Fries, *Hydnum* Fries, and *Thelephora* Fr. members of different subclasses, and *Peziza* Fr. members of different classes), the needless mortality among names could be very extensive. The remedy is to limit the Rule to its needed application before someone becomes too conscientious—in the manner of O. Kuntze.

Proposal 2: after "individual" insert the sentence, "Names given to *Lichenes* are considered to apply to the fungal component only."

Argument: Art. 64 as it stands invalidates most names given to lichens—all that antedate the Schwendenerian hypothesis. Commonly the algal component has a name proper to it; the fungal component rarely or perhaps never has. In the majority of lichens the fungus is the more conspicuous element, and supplies the majority of the characters used in taxonomy; unless new names are to be given to these fungi, the ones already applied to lichens should not be invalidated.—DONALD P. ROGERS, NEW YORK BOTANICAL GARDEN.

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XL

NOVEMBER-DECEMBER, 1948

No. 6

NOTES ON CERTAIN GASTEROMYCETES, INCLUDING TWO NEW ORDERS¹

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These notes have for the most part grown out of the study of type and other material at the New York Botanical Garden² and a certain few other types loaned from elsewhere.

HYMENOGASTRALES

1. *Gasterellaceae* fam. nov.

Fructificationes parvissimae, depresso-globosae, epigeae; gleba uniloculata; sporis brunneis, verrucosis.

Fructifications very tiny, depressed globose, epigeous; campanulate development; gleba finally uniloculate, but at times with one circle of cavities formed by vertical, centripetal invaginations which reach the center forming a false columella; cavities lined with a basidial hymenium; spores verrucose, dark.

Type genus: *Gasterella* Zeller and Walker.

This family is erected to take the two uniloculate genera, *Gasterella* and *Gasterellopsis*, while *Protogaster* with coralloid development and smooth spores is retained in *Protogasteraceae*.

¹ Published as Technical Paper No. 535 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany and New York Botanical Garden, Cryptogamic Herbarium, co-operating.

² Reference to the New York Botanical Garden Herbarium hereafter in this paper will be by initials only, N.Y.B.G.

* News has just been received that Dr. Zeller died from coronary thrombosis Nov. 4, 1948.

2. *CORDITUBERA* Hennings in Engler, Bot. Jahrbücher. 23: 557-558. f. V-8. 1897. [Syn. *Hoehneliogaster* Lohwag, Beih. z. Bot. Centralbl. 42 (Abt. 2): 299. 1926.]

We can see no particular reason for the genus *Hoehneliogaster* and thus it is being referred back to synonymy with *Corditubera* whence it came. The differences are more specific than generic. In *C. microspora* upon which Lohwag based his new genus, the basidia-containing lacunae are not quite filled with the netted reticulum of basidia-bearing hyphae, so that toward their centers are left slight cavities, whereas in *C. Staudtii* the lacunae are completely filled. Although the gleba is reddish in one species and yellowish in the other, the genus *Corditubera* is more closely related to *Leucogaster* than to any other genus.

3. The description of *Hydnangium* Wallroth given by Cunningham³ as emended by Ed. Fischer (Nat. Pflanzen-familien 7a: 30-31. 1933) is very misleading, especially so where he indicates the "columella dendroid, arising from a well-developed, sterile base," whereas the original description by Wallroth definitely states that a sterile base is *not* present and makes no mention of a columella. Fischer's description too was misconstrued and erroneously stated; thus Cunningham thought he might have reason to relegate Cava's *Arcangeliella* to synonymy with *Hydnangium*. The facts are that Fischer presented informal taxonomic descriptions and actually pointed out a contrast between *Hydnangium* and *Arcangeliella*; that whereas in a species like *Hydnangium carneum*, the type of the genus, in which its primordial stages *only* have a columella, *Arcangeliella* retains the columella and pileate peridium to maturity.

After having collected and examined in the field and in the laboratory many specimens in both of these groups we are in agreement with Fischer on the relationships between *Arcangeliella* and *Hydnangium* and will abide by the published treatment of these two genera and *Octaviania*.⁴

³ Cunningham, G. H. The Gasteromycetes of Australia and New Zealand. See p. 63. 1942.

⁴ Mo. Bot. Gard. Ann. 23: 574-575; 602-605. 1936.

4. *Hydnangium nigrescens* sp. nov.

Fructificationes 2.5–4 × 1–3 cm. crassae, oblongae vel subglobosae, rhizomorpho affixae; superficiei glabra, molli, alba dein nigrescente; fibrillis paucis, ad superficiem inferiorem applanatis; peridio simplici, 130–150 μ crasso, siccato 45–60 μ crasso, hyphis hyalinis magnis circa 7.5–9 μ crassis implicatis composito, superficiei fuscata; gleba brunnea; locellis medii-magnitudinis; septis tenuibus, albis, hyphis compactis parallelibus compositis, circa 60–85 μ crassis (hymenio annumerato); basidiis 2-sporigeris; sporis subglobosis, 12–16 μ , pedicellatis, obscure brunneis, uniguttulatis, episporio crasso, verrucoso, verrucis magnis, 10–12 circumferentiam quamquam ornantibus.

Fructifications oblong to subspherical, about 2.5–4 cm. × 1–3 cm., attached by a single rhizomorph; surface soft, glabrous, with some innate veins especially below, white becoming black on oxidizing, drying fuscous black; peridium 130–150 μ thick when fresh, drying 45–60 μ thick, spongy, composed of large loosely woven, hyaline hyphae, about 7.5–9 μ in diameter, the surface hyphae dark, forming a slightly more compact rind; gleba brown, drying Prout's brown; cavities relatively large; septa white, drying papery thin, of compact, parallel hyphae, about 60–85 μ thick (including hymenia); basidia 2-spored; spores subglobose to slightly ellipsoid, sometimes with remains of the stout sterigma, 12–16 μ in diameter, dark brown with one large vacuole, episporium thick, rough with very large blunt verrucae, 10–12 per circumference.

Partially exposed on the ground in mixed woods. **Type** collected in Cornell Plantations along Fall Creek, east of Floriculture Gardens, Tompkins county, New York, C. T. Rogerson, No. 1615, August 18, 1947. (In Cornell University, Plant Pathology Herb. No. 37257, and also in Zeller Herb.)

H. nigrescens has spore markings like those of *H. purpureum* but the spores are larger. It also differs in peridial characters, especially color, thickness, and size of hyphae.

5. *Hydnangium vesiculosum* (Coker & Couch) n. comb. (Syn. *Gymnomyces vesiculosus* Coker & Couch, *Gasteromycetes of Eastern U. S. and Canada*, p. 23. 1928.)

Fructifications subspherical, about 1 cm. diameter, drying to about half size, attached by a few basal fibrils which spring from a narrow depression; surface light buff yellow (about Naples yellow), spongy; peridium at first thin, of delicate, loosely woven, yellowish hyphae, quite evanescent, nearly absent at maturity, ex-

posing the gleba in places; gleba pallid gray-brown (pale earthy buff), firm but pliable, not tough or elastic; cavities rather large and box-like, empty, about 0.4–0.8 mm. in diameter; septa about 0.17–0.2 mm. thick including the hymenia which are about $30\ \mu$ thick, composed of large bladdery cells (pseudoparenchyma) about $20\text{--}40\ \mu$ thick except for a very thin layer under the hymenium where the cells are small; basidia short, thick, 4-spored; sterigmata nearly half as long as the spore diameter; spores concolorous with gleba, spherical, $7.5\text{--}10\ \mu$ including the strong blunt spines, often stuck together in groups of 4, sometimes finely reticulate.

Type locality: Chapel Hill, North Carolina.

Habitat: Exposed by erosion on soil in frondose woods.

Distribution: Known from type locality only.

Illustrations: Coker & Couch, *Gasteromycetes of Eastern U. S. and Canada*. pls. 16, 17, 105, f. 17–19.

As stated previously there is no sound basis for retaining *Gymnomycetes* since all the species have a peridium, even though it may be evanescent. The same argument holds for the retention of *Chamonixia* as separate from *Gautieria* since most, if not all, species of the latter have an evanescent peridium.

The species *Gymnomycetes vesiculosus* is, therefore, transferred to *Hydnangium*. The spores are sculptured as described by Coker & Couch but they also have very fine reticulations which seem *not* to be at all raised (alveolate).

6. *Elasmomyces Rodwayi* (Masse) n. comb. (Syn. *Secotium Rodwayi* Massee, Kew Bull. Misc. Info. 1901: 158. 1901.)

A part of the type collection from the Herbarium of George Massee is at the N.Y.B.G. The spores are subglobose, distinctly echinulate with blunt spines and also reticulate with large meshes, practically hyaline, short pedicellate, and $9\text{--}11\ \mu$ in diameter.

7. *MACOWANITES* Kalchbrenner, Hedwigia 15: 115. 1876; Grevillea 10: 107. 1882. [*Macowania* Kalchbrenner, Gardeners' Chron., N.S. 5: 785. 1876, *non Macowania* Oliver in Hooker, Icon Pl. III 1: 49. 1870.—*Hypocharnum* Kalchbrenner, Gard. Chron., N.S. 6: 140. 1876. *nomen nudum*.]

As was indicated previously⁵ the spores of *Macowanites agaricinus* Kalchbr. are borne "asymmetrically on the sterigmata as in

⁵ Zeller, S. M., and C. W. Dodge, Ann. Mo. Bot. Gard. 23: 636–637. 1936.

the Hymenomycetes." This matter has just been studied again and the part of the type in the herbarium of the N.Y.B.G. proves to be part of an aberrant Agaric belonging perhaps to *Russula*. The spores are slightly ellipsoid, echinulate, pedicellate with the sterigmatal scar asymmetrical to the main axis of the spore. There is also pseudoparenchyma in the tramal tissues as well as in the pileate tissue. According to nomenclatural procedure the genus is rejected and other species which have been assigned to this genus are disposed of as follows:

MacOwanites magnus Parks has lactiferous ducts in the sterile tissues and is like *Arcangeliella* in general morphological development. It, therefore, becomes *Arcangeliella magna* (Parks) n. comb. and *M. alpinus* Zeller is recombined as *Elasmomyces alpinus* n. comb.

8. GYMNOGLOSSUM Massee, Grevillea 19: 97. 1891. (Syn. *Dendrogaster* Bucholtz, Hedwigia 40: 316-318. 1901.)

Study of a part of the type collection of *Gymnoglossum stipitatum* Massee in the herbarium of N.Y.B.G. reveals that Cunningham's³ reduction of *Dendrogaster* to synonymy is justified. The specimen examined has smooth spores, however, as Massee reported. They are $13-16 \times 6-8.5 \mu$, definitely citriform with a very small apiculus and sometimes with a short pedicel. Since *Dendrogaster* is thus reduced to synonymy the following new combinations are necessary, *Gymnoglossum majus* (Z. & D.), *G. megasporum* (Z. & D.), *G. cambodgense* (Patouillard), *G. candidum* (Harkness), *G. radiatum* (Lloyd), *G. connectens* (Bucholtz), *G. utriculatum* (Harkness), *G. globosum* (Harkness), *G. foetidum* (Coker & Couch), *G. olivaceum* Zeller, and *G. elasmomycetoides* Zeller.

HYSTERANGIALES

1. PROTUBERA Möller, in Schimper, Botanische Mitteilungen aus den Tropen 7: 10-22: 145. T VI, f. 1-10. 1895. (Syn. *Protophallus* Murrill, Mycologia 2: 25. 1910.)

There are two dried collections of *Protuberia Maracuja* Möller in the Cryptogamic Herbarium of the N.Y.B.G., both ex Farlow

³ l.c. See p. 71.

Herb., Harvard University and both collected by Rev. J. Rick as follows: Rick Fungi Austro-Americani, No. 36, taken at Sao Leopoldo, 1904, and Rick Expeditions in Brazil, No. 136, taken at Parecy Novo, Rio Grande do Sul, 1928. Both collections answer very closely Möller's description of *P. Maracuja*. After the fructifications were soaked in water for 10–12 hours the character and organization of the essential parts were readily observed. There were found but two minor differences between *Protubera* and *Protophallus* Murrill. The one is hypogeous, the latter epigeous, and the former has a columella branched from the base or point of attachment. In the two species of *Protophallus* the columella is simple, extending to the center of the fructification and from its summit the tramal plates radiate. Such differences are more specific than of generic rank and it would seem wise to unite the two genera and transfer the two species of *Protophallus* to *Protubera* as *Protubera jamaicensis* (Murrill) n. comb. and *P. brunnea* Zeller n. comb.

P. Maracuja and *P. brunnea* are quite closely related but they differ in color, and characters of columella and spores. *Protubera* and *Calvarula* will be retained as members of the family *Protophallaceae*.

PHALLALES

1. CLATHRUS Persoon, Syn. Meth. Fung. p. 241. 1801. (*Dycticia* Rafinesque in Desraux, Jour. Bot. 2: 176. 1809.—*Clathrus* sect. *Clethria* Fries, Syst. Myc. 2: 288. 1823.—*Ileodictyon* Tulasne, Ann. Sci. Nat. Ser. III 2: 114. 1844.—*Clathrella* Fischer, in Engler & Prantl, Nat. Pflanzenfam. 1: [Ab. 1**] 284. 1898.)

It is with some hesitancy that *Clathrella* Fischer is placed in synonymy with *Clathrus*, but there seems to be only one real distinction besides size to separate them, namely the longer meshes on the sides compared with those over the top of the receptacle of *Clathrella* while in *Clathrus* these meshes are more or less the same size throughout. Also the drawn-out stem-like base in some individual plants of *Clathrella* is not a constant character and may be as conspicuous in some individuals of *Clathrus* as in individuals of *Clathrella*. It may or may not be present in all plants of a single collection according to Cunningham.³

³l.c.

Ileodictyon was proposed by Tulasne to receive those species of *Clathrus* with tubular, not chambered, arms of the receptaculum. Here again, Cunningham has found that "the type species, *Clathrus cibarius*, contains plants with both tubular and cellular arms, small plants as a rule possessing tubular, and large ones, cellular arms." Cunningham is, therefore, followed in grouping species of *Clathrella* and *Ileodictyon* under the genus *Clathrus*.

2. *LYSURUS* Fries, *Systema Mycologicum* 2: 285–286. 1823. (*Anthurus* Kalchbrenner & MacOwan in Kalchbrenner & Cooke, *Australian Fungi*, *Grevillea* 9: 2. 1880.—*Aserophallus* Lep. & Mont. *Ann. Sci. Nat.* [Ser. 3] 4: 360. 1845.—*Mycopharus* Petch, *Brit. Myc. Soc. Trans.* 10: 281. 1926.—*Pharus* Petch, *Bot. Gard. Peradeniya*, *Ann.* 7: 59. 1919.—*Lysurus* sect. *Desmaturus* Schlechtendal in *Linnaea* 31: 180. 1861–62.)

Peridium duplex, outer layer thin and furfuraceous, inner thick, gelatinous, remaining at the base of the stem as a volva at maturity; receptacle a hollow cylindrical or flaring stem, carrying at its summit a number of simple arms apically free or united organically or by a delicate membrane, continuous with or somewhat distinct from the stem below; gleba borne on the inner surfaces and sides of the arms, olivaceous, mucilaginous, foetid; spores smooth, phalloid.

Type species: *Lysurus Mokusin* (Pers.) Fries.

The similarity between *Lysurus* and *Anthurus* has been discussed by Cunningham.³ Early workers as well as workers of today have not been certain of the taxonomy of these two genera, and since the type specimen upon which Kalchbrenner & MacOwan erected the genus *Anthurus* no longer exists and the original description of *Anthurus* could just as well apply to *Lysurus*, *Anthurus* is reduced to synonymy. We cannot agree with Cunningham that *Pseudocolus* Lloyd is synonymous with *Anthurus* or *Lysurus*. It stands apart as a genus in which the arms of the receptacle are long and narrow and united at the apex.

The species that have been assigned either to *Anthurus* or *Lysurus* have at one time or another usually been referred to both genera so there are few if any new combinations to be made by the union of the two under the name *Lysurus*. There are three spe-

³l.c. See pp. 101 and 104.

cies in North America, namely, *Lysurus Gardneri* Berkeley, London Jour. Bot. 5: 535. 1846, *Lysurus Mokusin* (L. ex Pers.) Fries, Syst. Myc. 2: 286. 1823, and *Lysurus pusillus* Coker, Mycologia 37: 781-783. 1945.

3. *KUPSURA SPHAEROCEPHALA* Lloyd, in Lloyd, Myc. Writ. 7: 1303. f. 2903-2904. Oct. 1924.

The type specimen (No. 24893) (kindly loaned by Dr. J. A. Stevenson), upon which Lloyd based the genus *Kupsura*, proves to be a very mature, softened specimen of *Simblum sphaerocephalum* Schlechtendal. When the specimen was fresh the stem of the receptacle had been crowded up into the head and it was dried in that condition. This was misleading to Lloyd who took the brown interior (receptacle) of the head to be brown glebal tissue. Misleading also is a portion of the volva which remained over the top of the latticed head. *Kupsura* Lloyd thus becomes a synonym of *Simblum*.

4. *STAHELIOMYCES CINCTUS* Ed. Fischer, Mitteil. Nat. Ges. in Bern 1920: XXXV and 137. 1921.

This genus has been reported previously from British and Dutch Guiana. There is a very good pencil sketch made by A. F. Porter, Decorah, Iowa, of a specimen about 9 inches tall and 1.5 inches broad which he found in a dense jungle forest on the Bobanaza River, near Saryacu [Curicucha], Eastern Ecuador. The sketch is in the N.Y.B.G. Herb. This extends the known range fairly well across northern South America. There is no mistaking the identity of the fungus from which the sketch was made.

Somewhat more difficult, however, is the identification of the fungus from which was made a water-color painting to be found in the Ellis Collection at the same herbarium. Under the sketch in Ellis' handwriting is "*Corynites Ravenelii* B. & C. from Miss Lizzie Berk Hancock, Burlington, N. J., Autumn, 1881." The illustration is of a volvate fungus with reddish receptacle like a *Mutinus* (Syn. *Corynites*) but with the gleba in a zone around the receptacle some space below the apex, the tip of which had evidently been broken off. There was not the usual constriction of the receptacle at the glebal band as in *S. cinctus*. The plant evidently had all of the characters of *Staheliomyces* but if the re-

ceptacle was red and there was no constriction of the latter at the glebal zone it may have been a distinct species. There is a question, therefore, as to the species; and was the painting made from a specimen actually collected in New Jersey?

5. *ITAJAHYA GALERICULATA* Möller, in Schimper, Bot. Mitt. aus den Tropen 7: 79. pl. 5. 1895.

Our knowledge of the genus *Itajahya* in the United States is greatly enhanced by the careful field notes and compilations of the late Dr. W. H. Long⁶ and his co-operators. He has reported the genus from Texas, New Mexico, and Arizona. In the herbarium of the N.Y.B.G. is a specimen of *Itajahya galericulata* collected by E. Bethel near Denver, Colorado. There are no further collecting data with the specimen.

LYCOPERDALES

1. The *Lycoperdaceae* of the past has been more or less a catch-all for any gasteromycete having capillitium and so it seems best to divide the family as presented by Fischer (1933) into more unified entities. The genera with stromate fructifications have been referred to the new family *Broomeiaceae* and those with spiny capillitium are included in the new family *Mycenastraceae*.

Broomeiaceae fam. nov.

Fructificationes stromatae ovoidae vel subglobosae; peridio duplici, exoperidio tenui subevanescenti, endoperidio membranaceo, stomatibus; capillitio simplici.

Fructifications single or many on a stroma, mostly ovoid, hemispheric or subspheric; exoperidium thin, wholly or partly disintegrated at maturity, endoperidium papery or thickish, laid bare at maturity, opening by an apical pore; capillitium present, threads more or less symmetrical, simple.

This family was created to care for the three genera with stromate fructifications, *Broomeia*, with a stalked stroma, *Diplocystis*, with a resupinate or patellate stroma, and *Lycogalopsis* in which the fructifications are borne singly on a stroma. Fischer (1933)

⁶ Long, W. H., and D. J. Stouffer. The genus *Itajahya* in North America. *Mycologia* 35: 620-628. *Illus.* 1943.

questioned whether these genera should be included in the Lycoperdales at all, but I am referring the *Broomeiaceae* to this order.

Mycenastraceae fam. nov.

Fructificationes magnae, subglobosae; peridio duplici, exoperidio crasso, spongioso, glabro vel areolato, endoperidio crasso coriaceoque vel tenui tenacique, demum irregulariter dehiscenti; capillitio ramoso, spinoso; sporis globosis vel ellipsoideis, verrucosis.

Fructifications large, subglobose; peridium duplex, exoperidium thick, spongy, smooth or areolate, endoperidium thick and leathery or thin and membranaceous; capillitium branched with short, pointed, spine-like branches; spores globose to ellipsoid, verrucose.

This family is proposed to contain the genera *Mycenastrum* and *Calbovista*.

2. *LYCOPERDON ALBINUM* Cooke, in Masee, Jour. Roy. Microsc. Soc. 1887: 723. Oct. 1887.

Lloyd[†] must have applied from memory Cooke's name of this fungus to Porto Rican collections made by John A. Stevenson and used the misnomer *L. albidum* instead of *L. albinum*. A slide (No. 58690) in the Lloyd Herbarium prepared by Lloyd from the type of *L. albinum* Cooke has been studied and it appears that Mr. Lloyd was justified in referring the Porto Rican collections to this species. Lloyd and Cooke, however, made the same mistake; the spores are finely asperate instead of smooth. An emended description follows:

Fructifications 4–12 mm. in diameter, subspherical to pyriform, sessile, yellowish with a whitish bloom, smooth to slightly checkered by tiny cracks; peridium thin, somewhat farinose, yellowish; gleba yellowish then turning grayish; capillitium scanty, hyaline, slender, flaccid; spores spherical to somewhat irregular, slightly asperate, almost hyaline, 2.5–4.2 μ .

Type locality: Brazil, South America.

Habitat: On rotted wood or on humus in soil.

Distribution: Puerto Rico and Brazil.

Illustrations: Lloyd, Myc. Writ. 5: 582. f. 822. 1916.

[†] Lloyd, C. G., Myc. Writ. 5: 582. f. 822. 1916.

3. *COILOMYCES SCHWEINITZII* Berk. & Curtis.

A study of the type of this genus and species, which is to be found in the Herbarium at N.Y.B.G., verifies Fischer's⁸ assumption that this genus was based on a collection of *Geastrum mirabile* Mont. from Surinam. The central cavity to which the authors referred and upon which the generic name was based is where the relatively large columella had collapsed. The Berkeley and Curtis specific name predates Montagne's name and necessitates the following new combination:

***Geastrum Schweinitzii* (Berk. & Curtis) Zeller n. comb.**

Coilomyces Schweinitzii Berk. & Curtis, Jour. Acad. Nat. Sci. Philadelphia (Series 2) 2: 279-280. 1853.

Geastrum mirabile Mont., Ann. Sci. Nat., Ser. IV 3: 139-140. pl. 6, f. 8. 1855.

Geaster papyraceus B. & C., Am. Acad. Arts & Sci. Proc. 4: 124. 1858.

Geaster lignicola Berk., Linn. Soc. Bot. Jour. 18: 386. 1891.

Geaster caespitosus Lloyd, Myc. Writ. 2: 315. pl. 100. 1907.

4. *Bovistella atrobrunnea* sp. nov.

Fructificationes depresso-globosae vel turbinatae, superne saepe collapsae, 3-4 cm. crassae, 2-3 cm. altae, rhizomorphis affixae; superficie impolita leves vel leniter furfuraceae vel leniter rimosae, siccitate obscure brunneae; exoperidio tenui, fragili, superne squamis facile separabilibus discedisque vestito; endoperidio tenuissimo papyraceoque, impolito- vel nitido-glabro, apice osculo laciniato dehiscente; basi sterili cellulosa, convex inscula, nitido-brunnea septis instructa; gleba pulverulenta obscure vinaceo-brunnea vel obscuriora; capillitio discreto, longo, paulo ramoso, terminalibus longis attenuatis tenuioribusque, obscure brunneo; sporis brunneis, sphaericis, verrucosis, 5-8 μ , longe pedicellatis.

Fructifications oblate spheroid to turbinate, often collapsed above, 3-4 cm. broad, 2-3 cm. high, with a prominent attachment but not particularly radicate; surface dull, smooth to somewhat furfuraceous, somewhat rimose, dark brown (dry); peridium duplex, exoperidium thin, brittle, breaking up into small plates which easily separate and fall away; endoperidium very thin, papery, dull to shiny, a little lighter colored than the exoperidium, dehiscing by a torn irregular apical pore; sterile base prominent, somewhat convex above, occupying one-third to one-half of the lower portion of the fructification, of large cells, separated by thin, shiny, metallic-brown walls; gleba pulverulent, dark vinaceous brown or darker; capillitium free, long, slightly branched

⁸ Fischer, Ed. Gasteromycetes, in Engler and Prantl, Die Nat. Pflanzenfam. 7a: 1-122. 1933. (See p. 76.)

or simple, with long tapering narrow (or even thread-like) terminals, dark brown, somewhat uneven; spores brown, spherical, verrucose, 5–8 μ , with a hyaline pedicel up to 30 μ long which is easily broken away.

On the ground, Ann Arbor, Michigan, October 6, 1936, *A. H. Smith*, 5048a, **type** (in U. Mich. Herb., portion in Zeller Herb.).

Bovistella atrobrunnea differs from other species of the genus in the very dark gleba, the spherical, verrucose spores and particularly in the capillitium. The latter is nearly simple but, now and then, dichotomously branched and the branches or terminals are tapering or drawn out to very long, narrow thread-like filaments very much narrower than the main stem of each unit of capillitium. It is named for the very dark brown gleba.

5. *Morganella* gen. nov.

Fructificationes parvae, subglobosae; peridio duplici, apice ore dehiscente; gleba pulverulenta, capillitio tenuissimis membranisque ad basim radiantes; sporis sphaericis, coloratis.

Fructifications small, subglobose; peridium duplex, dehiscing by an apical stoma; gleba pulverulent, with capillitium and filmy membranes radiating from base to peridium; spores spherical, colored.

Type species *Morganella mexicana*.

Morganella mexicana sp. nov.

Fructificationes parvae, subglobosae, solitariae vel caespitosae, appendici radiciformi albo adfixae; peridio duplici; exoperidio furfuraceo, brunneo, in squamas furfuraceas fisso; endoperidio papyraceo-tenui, apice ore irregulari dehiscenti; basi sterili inconspicua, contextu flavido solidiusculo sine locello composito; gleba pulverulenta, pallido-grisea vel pallido-brunnea, tenuissima membrana et capillitio sporisque composita; membranae hyalinae, ut bullatis sacculis instructis, atque notis lunulatis appareant; capillitio hyalino, laevi, paulo ramoso; sporis rubro-brunneis, sphaericis, paulo verrucosis, 3.7–4.3 μ .

Fructifications from a white, mycelial rhizomorph, small, subglobose, single or caespitose; peridium duplex; exoperidium furfuraceous, becoming separated over the endoperidium in the form of tiny furfuraceous squamules, bay or natal brown becoming hair brown; endoperidium thin, papery, dehiscing by an apical irregular rupture; gleba with a very slight sterile base which is of yellowish solid tissue without apparent chambers, fertile portion pulverulent, pale gray, becoming light chocolate brown, composed at maturity

of very filmy membranes (in which capillitium is enmeshed) radiating from the base to the endoperidium and spores; membranes hyaline, with tiny pockets and swellings appearing as crescent-shaped markings; capillitium threads (embedded in the filmy membranes) hyaline, smooth, very slightly branched; spores reddish brown, spherical, slightly verrucose, $3.7-4.3\ \mu$.

Type locality: Near Guaymas, Sonora, Mexico.

Habitat: In wet places among moss and leaves.

Distribution: New Jersey, Mexico, Panama, and Colombia.

Specimens examined: New Jersey: Newfield, Aug. 1887, J. B. Ellis, No. 5013 (in N.Y.B.G. Herb.); Mexico: Sonora, near Guaymas, *Thomas H. Macbride*, type (in Morgan Herb. at University of Iowa); Panama: Canal Zone, Ft. Sherman Area, G. W. Martin, No. 6183; and South America: Colombia, Sierra Nevada de Santa Marta, Dept. Magdalena, Hacienda Cincinnati, G. W. Martin, No. 3440 (both in Mycological Collections of the University of Iowa).

In the N.Y.B.G. Herbarium there is correspondence between Thomas H. Macbride, J. B. Ellis, and A. P. Morgan relative to the collection of this fungus taken by Macbride in Mexico and to which the collector had given the tentative name *Lycogala mexicanum*. The following letter from A. P. Morgan to Macbride seems worth quoting:

"That is a queer thing you sent me, but I think it is a puffball —*Lycoperdon*. I cut it in two and scanned it internally and externally. The peculiar membrane with disk-like markings certainly resembles the membrane of *Myxomycetes*, it is very thin and iridescent, but the spores are those of a puffball. I managed to get off some bits of the rind, which I consider to be made up of true hyphae; I enclose them to you. The threads of the capillitium are unlike those of ordinary puffballs; but there are two puffballs that have similar threads and they have bits of membrane adhering to them in the same way; these are *Lycoperdon Curtisii* Berk. and *L. acuminatum* Bosc. Of course the membrane is not as marked a feature as in this specimen.

"But what is more, in cutting down through the peridium I arrived at a slender rooting mycelium, a thick strand of hyphae in it. Tear this up or mark it with the point of a knife blade and

you can see the hyphae comprising it plainly with about 500 diameter.

"I am quite sure that it is a *Lycoperdon*, but it is nothing I have ever seen before."

This truly is a most interesting Gasteromycete which belongs in the *Lycoperdaceae* or the *Mesophelliaceae*. Until more is known of the origin and development of the membranes in the fertile gleba it is placed in the *Lycoperdaceae* for convenience. It seems possible that the membranes mentioned above may originate from a very thin tramal tissue between the hymenia of the septa. The origin of the pockets and swellings in the membranes is beyond our present understanding.

Morgan's mention of membranes in the gleba of *Lycoperdon Curtisii* Berk. and *L. acuminatum* Bosc. is unexplainable unless he was dealing with very young specimens in which the tramal tissues are just breaking down. Such membranes, however, seem unrelated to such as found in *Morganella* at maturity.

At late maturity of *Morganella* the whole gleba disappears, leaving a more or less cupulate or discoid, empty peridium, exposing the silvery gray inner surface of the endoperidium.

6. *Radiigera cinnamomea* sp. nov.

Fructificationes 1-3 cm. crassae, depresso-globosae vel turbinatae, basi saepe radicans; superficie cinnamomea, squamis minutis, concoloribus, saepe apice conniventibus obsita; basis sterilis superne convexa inscula, inferne attenuata, cellulis parvis composita; columella conica vel subglobosa, mollo-spongiosa compacta, concolor; peridio duplici 0.5 mm. siccato; exoperidio squamoso; endoperidio suberoso, fragili siccato, 250-300 μ crasso; gleba cinnamomea, fasciculis hypharum capillitioque sporisque composita; capillitio ad columellam percursum radians hyalino molli, flexuoso, inaequali, 6-7 μ crasso; sporis pallidissime-brunneis, sphaericis, sparse-echinulatis, pedicellatis, 3.75-5 μ crassis, non guttulatis.

Fructifications 1-3 cm. diam., depressed globose to turbinate, with a slightly projecting basal attachment; surface dull cinnamon brown, with minute indistinct, concolorous, sometimes connivent scales; sterile base very slightly convex above, somewhat attenuate below, cells small but distinct, crowned by a broad subspherical to conical columella which occupies about one-half of the central portion of the fructification; columella concolorous with the gleba, composed of a compact, soft, pithy, homogeneous (?) tissue; peridium duplex, total drying about 0.5 mm. thick, exoperidium scaly

as described above, endoperidium corky, brittle, about 250–300 μ thick; gleba cinnamon brown throughout, composed of fascicles of hyphae and capillitium radiating from the surface of the columella to the inner wall of the endoperidium, readily separating from the former on drying; capillitium hyaline, various sizes of filaments up to 6–7 μ in diam., soft flaccid, sometimes with very filmy remains of hyaline membranes adhering; spores very dilute brownish, almost hyaline, spherical, minutely and sparsely echinulate, 3.75–5 μ , with a long flexuous (collapsed) pedicel, not guttulate.

Type locality: Near Philadelphia, Pa., collected by T. G. Gentry, type (in N.Y.B.G. Herb. and portion in Zeller Herb.).

Distribution: Known from type locality only.

SCLERODERMATALES

1. SCLERODERMA AUREA Massee in Cooke, Grevillea 18: 26. 1889.

One-half of a specimen in the N.Y.B.G. Herb. is labelled in George Massee's handwriting as follows: "Scleroderma aurea, Mass., New Guinea, TYPE." It is a small-spored form of *S. aurantium* Pers. The spores are globose, alveolate-reticulate-echinulate, 6.3–7.5 μ . The echinulae are quite long and acute.

2. SCLERODERMA COLUMNARE Berkeley & Broome, Ceylon Fungi No. 726.—*Areolaria columnaris* (Berk. & Br.) DeToni in Sacc. Syll. Fung. 7: 144–145. 1888.

A supposedly authentic specimen from Ceylon purchased from George Massee by the New York Botanical Garden is the short stipitate form of *Scleroderma verrucosum* Pers. Cunningham referred *S. columnare* to *S. Bovista* and Lloyd referred it to *S. cepa*. The latter is more nearly correct since the spores are not reticulate. The spores are bluntly echinulate, the peridium warted, and otherwise the specimen appears like *S. verrucosum*.

3. SCLERODERMA LYCOPERDOIDES Schw. Schrift. Naturf. Ges. Leipzig 1: 61. 1822.—*Scleroderma tenerum* B. & C. Cuban Fungi No. 512.

The type collection of *Scleroderma lycoperdoides* Schweinitz is in the Ezra Michener Collection, Vol. 17, Sheet No. 37 (U.S.D.A., Bureau of Plant Industry, Mycological Collections, Beltsville,

Maryland). The collection is labelled "No. 2242, Syn. Fung. *Scleroderma lycoperdoides* Schw., on the earth, Carolina (ex Herb. Schw.)." This specimen has spores with closely set acute spines and they average about 12-14 μ . It seems in every respect like *S. tenerum* B. & C.

4. *SCLERODERMA TUBEROIDEUM* Speg. Anal. Mus. Nac. Buenos Aires 16: 28. 1906.

An authentic specimen of this species sent by Spegazzini to the N.Y.B.G. proves to be *S. cepa* Pers.

5. *ASTRAEUS HYGROMETRICUS* (Pers.) Morgan is a cosmopolitan species throughout temperate climates. The species is quite variable in surface characters of the exoperidium. There are smooth, even glossy specimens, others that are rough and felty, while still others are fibrillose scaly. Otherwise there seems to be little variability except in size.

One collection taken by *O. E. Jennings*, in sand dunes, Presque Isle, Erie county, Pennsylvania (in Herb. Carnegie Museum, Pittsburgh), is a very smooth form to which a great deal of study has been given and merits further attention. The peridium has the same structure as that of typical *A. hygrometricus*, but when soaked in water the exoperidium does not dehisce along well-marked sutures radiating from the apex. On the contrary it cracks along irregular lines. If other collections may be found to show the same tendencies this form could hardly be included in *Astraeus*.

6. *Astraeus pteridis* (Shear) comb. nov. (*Scleroderma pteridis* Shear, Bull. Torr. Bot. Club 29: 451. 1902.—*Geastrum hygrometricum* Pers. var. *giganteum* Lloyd, Myc. Writ. 1: 68. f. 30. 1901.)

Most of the years since 1909 I have spent in western Oregon or Washington and have wondered why *Scleroderma pteridis* Shear could not be found. Recently a portion of the type was discovered at N.Y.B.G. It proved immediately to be an old acquaintance, the "Giant *Astraeus*" of the Pacific northwest. It is not unusual to find the buttons up to 2 inches in diameter. The largest expanded star observed was over 9 inches. Shear men-

tioned its similarity to *Scleroderma geaster*. It is in size only. The peridial characters are entirely distinct.

7. *CALOSTOMA MICROSPORUM* Atkinson, Jour. Myc. 9: 16. 1903.

Emended description:

Fructifications 4-7 cm. high; foot stalk 3-6 cm. \times 1-1.5 cm., cylindrical or ventricose or enlarged below, sometimes compressed, rarely two or more footstalks joined throughout the length; endoperidium ovoid, 10-15 mm. broad, slaty or bluish gray to warm brown, mouth stellate with 5-7 prominently raised teeth, vermilion colored on their inner faces; exoperidium separating into numerous small, hard, adherent warts covering the middle and lower surface of the endoperidium and usually entirely wanting toward the apex where the endoperidium is quite smooth; spore sac pure white; spores pure white, smooth, oblong, some rarely ellipsoid, $6-10 \times 3.5-5 \mu$. Type locality: Rugby, Tennessee.

The type collection was kindly loaned by Dr. H. M. Fitzpatrick, and Atkinson's description has been emended to conform with other descriptions presented here. There are to us three outstanding characters of the species: The small ellipsoid spores which show little variability in size and shape, the very prominently raised peristome, and the prominence of the exoperidium left as warts on the sides of the endoperidium. The spores and the spore sac are also characteristically pure white.

A collection by L. W. Nuttall taken from wet mossy banks in Fayette county, West Virginia, extends the range. This collection predates the collection of the *type* by nine years. It was distributed as No. 881, Flora of Fayette county, West Virginia, collected by L. W. Nuttall, March 25, 1893. In this collection the endoperidium is warm brown while in the type collection it is slaty or bluish gray.

8. *Sedeculaceae* fam. nov.

Fructificationes coriaceae, sine basi sterili nec radicibus; peridio crasso, superne coriaceo, inferne paene obsoleto dehiscentique; gleba pulverulenta, cum venis crassis, a peridio ad centrum vergentibus; sporis brunneis, breve pedicellatis.

Fructifications leathery, without sterile base or radicle; peridium thick, leathery above, almost obsolete and dehiscing below; gleba becoming powdery at maturity, with broad veins extending in-

ward from the peridium; spores brown, pedicellate or with sterigmatal scar.

From the specimens so far examined it is evident that *Sedecula* develops centripetally and has complete reduction of stipe and sterile base. There is practically no sterile tissue remaining over the lower surface of the fructification where the gleba is almost if not usually naked. The genus was first placed for convenience in the *Sclerodermataceae* (Mycologia 33: 212. 1941) merely because of the heavy, leathery peridium. The sterigmatal scar or pedicellate character of the spores indicates closer relationship to *Pompholyx* than to *Scleroderma*, but because of the evidence of centripetal development it is here placed in a family by itself. *Sedeculaceae*, so far containing *Sedecula* only, is referred to the Sclerodermatales.

NIDULARIALES

1. SPHAEROBOLACEAE. The chief recent contributions to our knowledge of this family have been made by Walker⁹ and Greis.¹⁰

Walker has found two distinct types, *Sphaerobolus stellatus* Tode and *S. iowensis* Walker. Both of these species have the lacunar type of glebal development in which large buffer cells or space formers expand the glebal chambers before basidia are formed. In *S. stellatus* the basidia grow into and fill these spaces forming nests of basidia, as in the *Melanogastraceae* and *Sclerodermataceae*. (See Zeller, S. M., Developmental morphology of Alpova. Oregon State College Monographs in Botany No. 2. illus. 1939.) In *S. iowensis* the basidia form hymenial linings to otherwise hollow chambers. There are other differences. There is a gelatinous layer in the peridium in *S. stellatus*, not in *S. iowensis*, while the gleba of the latter dries soft or gluey and gelatinous and that of *S. stellatus* dries firm and hard.

Greis found a form of *S. iowensis* in Europe which he called forma *europaea*. In addition he has described the genus *Nidulariopsis*. The fruiting body here rises from a mycelial cord and

⁹ Walker, Leva B. Development and mechanism of discharge in *Sphaerobolus iowensis* n. sp. and *S. stellatus* Tode. Jour. Elisha Mitchell Sci. Soc. 42: 151-178. pl. 16-25. 1927.

¹⁰ Greis, H. *Nidulariopsis melanocarpa* Greis nov. gen. nov. spec. und eine neue Form von *Sphaerobolus iowensis*. Hedwigia 75: 255-266. illus. 1935.

the gleba is like that of *Sphaerobolus iowensis* with cavities lined with an even basidial hymenium. He says a special peculiarity is that the middle layer of the peridium consists of rounded brownish cells with thick walls, and this layer does not reach across the summit of the fruiting body. But this peculiarity was recognized by Lohwag¹¹ who believed that this layer indicates a cup-shaped primordial (tramal cup) development. Walker's illustrations of *S. iowensis* (Pl. 17, f. 11 & 13) show this interrupted middle layer very plainly.

It appears that any differences between *Sphaerobolus iowensis* Walker and *Nidulariopsis melanocarpa* Greis are merely specific rather than generic and that the two should be included in the same genus *Nidulariopsis*. In this arrangement then *S. iowensis* becomes *Nidulariopsis iowensis* (Walker) n. comb.

Since the two genera are so specialized in peridial structure and discharge of the gleba they are retained in the same family, *Sphaerobolaceae*.

Fischer (1933) referred this family to the Sclerodermatales as a special family. Its closest relationship in this order is to the *Calostomataceae*, because of the similarity in discharge of gleba. However, in *Calostoma* the spore sac is inverted and ejected through the stoma after the gleba has become a powdery mass, mostly spores. It would seem more reasonable to follow the usual procedure and include the *Sphaerobolaceae* with the *Nidulariaceae* in the Nidulariales.

PODAXALES

1. *Secotium albipes* sp. nov.

Fructificationes turbinatae, stipitatae; pileo circa 4 cm. alto, 5 cm. crasso, subglobozo, inferne a stipite inseparabili; superficie laevi, glabra, viscida, rubra, siccitate obscuriore; stipite 1-1.5 cm. longo, sursum crasso inferne tenuiori, solido vel farcto, superficie siccitate alba, interne obscuriore, superne in columellam crassam percurrentem procurrente, peridio circa 650 μ crasso (sicc.), prosenchymate gelatinoso composito, siccitate duro obscuroque; gleba obscure brunnea, locellis parvis; basidiis 1-, 2-, et 4-sporigeris; sporis brunneis, ellipsoideis, laevibus vel subrugulosis, guttulatis, 6-8 \times 12-18 μ ; cystidiis magnis conicis hyalinis.

¹¹ Lohwag H. Die Homologien im Fruchtkörperbau der höheren Pilze. I und II. *Biologia generalis* 2: 148-182, 575-608. 1926.

Fructifications turbinate, stipitate, up to 4 cm. tall and 5 cm. wide; pileus subglobose; smooth, glabrous, viscid, red, drying dark mineral red, not breaking from the stipe below; stipe short, 1-1.5 cm. long, broad above and tapered sharply downward, solid, drying dark within but whitish on the surface; columella percurrent, broad, but broader above and below; peridium about 650μ thick (dry), of gelified, prosenchymatous tissue, drying dark and hard; gleba dark brown, locules small; basidia 1-, 2-, and 4-spored; spores brownish, narrowly ellipsoid, smooth but with occasional raised spots, guttulate, $6-8 \times 12-18\mu$; cystidia large, conic, hyaline.

On the ground in rich forest duff.

California: Butte county, Merrimac, *Thelma Norman*, Nov. 9, 1932, **type** (in N.Y.B.G. Herb.).

The above collection had been labeled "*Secotium erythrocephalum* Tulasne." This drew our attention again to the original description of the latter in which Tulasne describes the stipe as 'white.' Cunningham has described the stem as 'bright yellow' and several collections from New Zealand in American herbaria show yellow slender stipes.

Secotium albipes differs from *S. erythrocephalum* in other respects, however, than in stipe color. The edge of the peridium (pileus) does not readily separate from the stipe below as in *S. erythrocephalum*, which not only separates but often the edge is turned back (*repandus* Tulasne), thin, and coriaceous. The latter is doubtless a part of the fundamental veil separating from the stipe. The dried plants of *S. albipes* are short and stout whereas those of *S. erythrocephalum* are slender and tall. The spores of the latter are smooth and those of *S. albipes* are smooth but have a tendency to slight roughness and are definitely guttulate. *S. albipes* is similar to *S. tenuipes* Setchell in color of the peridium but the latter has a brown stipe and the spores are dark brown, rough and citriform as in those of *Hymenogaster*.

2. SECOTIUM NUBIGENUM Harkness.

A recent note¹² indicated the loss of the type of this species, but in the N.Y.B.G. there is a collection with the label in Harkness' handwriting as follows: "*Secotium nubigenum* Hks., on logs of *Pinus contorta*, Summit of the Sierra Nevada." An additional

¹² Zeller, S. M., *Mycologia* 33: 210. 1941.

note that accompanied the original description is "7000 ft." Since this specimen was collected by Harkness at the type locality I have taken the liberty to label it "type." Unless Harkness distributed type material elsewhere, this at least is the only collection so authentic.

3. SECOTIUM SESSILE Massee and Rodway, in Rodway, Roy. Soc. Tasmania, Proc. 1911: 31. 1912. (*Elasmomyces sessile* Rodw. Roy. Soc. Tasmania, Proc. 1924: 8. 1925.)

A part of the type of the above is in the N.Y.B.G. Herbarium. The packet containing the collection is inscribed with the following in George Massee's handwriting: "*Secotium sessile* Mass. & Rodw., Tasmania, Rodway 649. type." The specimens are in every particular the same as *Elasmomyces Mattirolanus* Cavara, with which it becomes synonymous.

TWO NEW ORDERS

The genera of the Gasteromycetes for the most part fall into the older, established, and quite generally accepted orders, as follows:

1. THE HYMENOGASTRALES, which include mostly hypogeous species that retain the original glebal structures to maturity. Such genera have been assigned to the families *Protogasteraceae*, *Gasterellaceae*, *Melanogasteraceae*, *Rhizopogonaceae*, *Hymenogasteraceae*, and *Hydnangiaceae*. They contain about twenty-two genera.

2. THE HYSTERANGIALES include hypogeous or epigeous species with gelatinous or cartilaginous tissues and smooth, ellipsoid spores (phalloid) but without a so-called receptacle. They have been assigned to the families *Hysterangiaceae*, *Protophallaceae*, and *Gelopellaceae*. The three families contain seven genera.

3. THE PHALLALES are divided into the three families—*Claustulaceae*, *Phallaceae*, and *Clathraceae*. The species here have bacillar spores, the peridium a volva with at least one gelatinous layer (and that layer interrupted by sutures of fundamental tissue in the *Clathraceae*), and the gleba variously dispersed or elevated on a pseudoparenchymatous stem, or a clathrate or hollow structure known as the receptacle. There are about twenty genera referred to this order.

4. THE LYCOPERDALES, which include the "puff ball" types of fructifications having ordinary hyphal tissues that are not gelatinous or cartilaginous, the gleba disintegrating into a powder or into small hollow peridioles at maturity, and with a capillitium (except in *Arachniaceae*). The species here are assigned to the families *Arachniaceae*, *Broomeiaceae*, *Mycenastraceae*, *Lycoperdaceae*, *Mesophelliaceae*, and *Geastraceae*. These include about twenty-five genera.

5. THE SCLERODERMATALES that include species with heavy peridial and other tissues, and gleba pulverulent at maturity; basidia symmetrically distributed or in nests or cavities arising through the dissolution of the tissue, at least without a well organized hymenium (except possibly in *Batarrea*). The order has been divided into the families *Sclerodermataceae*, *Pisolithaceae*, *Glischrodermataceae*, *Sedeculaceae*, *Astraeaceae*, *Tulostomataceae*, and *Calostomataceae*. These families include seventeen genera.

6. THE NIDULARIALES, which are the distinctive "Bird's Nest" fungi and need no description here but are divided into the two families *Nidulariaceae* and *Sphaerobolaceae*, including six genera.

7. THE PODAXALES, the species of which have a percurrent columella or stem reaching to the summit of the fructification; peridium left at maturity in part as a pileus or volva, or annulus on the stem; gleba at first with hymenium of basidia covering the walls of chambers or pores or lamellae, persistent or pulverulent; spores colored. The order has two families, the *Secotiaceae* and the *Podaxaceae*, containing seven genera.

This treatment of the Gasteromycetes leaves four genera to be considered. These are *Clathrogaster*, *Gasterosporium*, *Gautieria*, and *Tremellogaster*. *Tremellogaster* and *Clathrogaster* may readily be placed in the same family because of their peridial and glebal characters. The peridia of these two genera are composed of at least two layers. The outer layer is of filamentous, fundamental tissue, forming a thin rind. Under this there is a thick gelatinous layer. In this layer the hyphae are far apart but are dispersed through a thick gel. This gelatinous layer is interrupted here and there by more or less radial plates (sutures) of more compactly interwoven hyphae, the same as the outer layer, and connecting the latter with the gleba, much as in the family *Protophallaceae*.

and in the early button stages of the *Clathraceae*. In the peridium of *Tremellogaster* these sutures are more numerous and complicated than in *Clathrogaster*.

The peridium of *Gastrosporium* as previously reported¹³ is very similar to that of the *Gelopellaceae* and to the volva in the button stages of the *Phallaceae*. The glebae of *Gastrosporium* and *Tremellogaster* are pulverulent at maturity. Whether the gleba of *Clathrogaster* retains its original structure to maturity is not known, but in other respects it is similar enough to *Tremellogaster* to be included in the same family with it. The type material of *Clathrogaster* was originally, at least, kept in preservative and the development of the gleba was arrested as at the time of collection. All of these genera, however, have spherical, echinulate or sculptured spores and, therefore, do not belong in the series of genera with phalloid spores, such as those of the *Hysterangiales* and *Phallales*.

To judge only from the gelatinous or cartilaginous character of the tissues in *Gautieria* this genus should take its place somewhere in the *Melanogastraceae*, *Hysterangiaceae*, or possibly the *Sclerodermatales*. Glebal characters, however, make it incompatible with the *Melanogastraceae* and the *Sclerodermatales* and the spore characters would keep it out of the *Hysterangiaceae*. One might stretch a point and place the genus *Gautieria* with the *Hymenogastraceae* having like spores, but it does not otherwise belong.

Two new orders are consequently proposed to receive these genera. The first, the **Tremellogastrales**, contains two families; the **Tremellogasteraceae** n. fam., including the genera *Clathrogaster* Petri and *Tremellogaster* Ed. Fischer, and the *Gastrosporiaceae* Pilat, including the one genus *Gastrosporium* Mattirollo. The second new order, the **Gautieriales**, contains the **Gautieriaceae** n. fam. with the one genus *Gautieria* Vittadini.

TREMELLOGASTRALES n. ordo

Fructificationes hypogaeae vel epigaeae, sessiles; peridio duplici, strato externo hyphis intricatis composito, strato interno gelatinoso continuo vel laminis radiantibus interrupto investitoque; gleba primo carnosa, dein pul-

¹³ Zeller, S. M. New and noteworthy Gasteromycetes. *Mycologia* 31: 1-32. 1939 (see p. 17).

verulenta; columella simplici vel dendroidea, vel deficienti; sporis sphaericis, echinulatis vel verrucosis.

Fructifications hypogeous or epigeous, mostly sessile; peridium of two or more layers, the outer of fundamental tissue, the inner of a gelatinous nature, continuous or interrupted by sutures of fundamental tissue; gleba centripetally developed, pulverulent at maturity; columella simple or wanting; spores spherical, echinulate or verrucose.

KEY TO THE FAMILIES OF THE ORDER TREMELLOGASTRALES

- I. Peridium with a gelatinous inner layer interrupted by sutures of fundamental tissue; spores spherical, echinulate or rough. .*Tremellogasteraceae*
- II. Peridium with a simple gelatinous inner layer, not interrupted by sutures; spores spherical, minutely verrucose.....*Gastrosporiaceae*

A. Tremellogasteraceae n. fam.

Fructificationes hypogaeae vel epigaeae, sessiles; peridio duplici, strato externo hyphis intricatis composito, strato interno gelatinoso laminis radiantibus interrupto investitoque; gleba ochracea vel flavida, primo carnosae, dein pulverulenti; columella dendroidea vel deficienti; basidiosporis sphaericis, echinulatis vel cristatis.

Fructifications epigeous or hypogeous, subglobose, sessile; peridium of two or more layers; exoperidium of fundamental tissues; endoperidium of one or more layers of gelatinous tissue interrupted by plates of fundamental tissue; gleba developing centripetally from hyphae from the margins of the medulla of the primordium, pulverulent at maturity; capillitium rudimentary; columella sometimes present; spores spherical, echinulate or cristate.

KEY TO THE GENERA OF THE TREMELLOGASTERACEAE

- I. Tramal peridium very thick, with branched sutures of fundamental, filamentous tissue dividing the gelatinous portion of the inner peridium radially and into two more or less definite layers; spores spherical, echinulate*Tremellogaster*
- II. Tramal peridium thinner, of one gelatinous layer interrupted by more or less simple, radial sutures; spores spherical, echinulate and ridged, or ridged only.....*Clathrogaster*

1. TREMELLOGASTER Ed. Fischer, in Mitteil. Naturf. Ges. Bern 1923: 49-56. 1924.

Emended description: Fructifications epigeous, subglobose; surface drying coarsely flattened-tuberculate; peridium very thick, up

to $\frac{1}{6}$ the diameter of the sporocarp, with an outer layer of thick-walled, sclerotoid hyphae bordered internally by thin-walled, hyaline periclinal hyphae, a middle layer is brownish, gelatinous, reticulately divided by lighter colored nongelatinous, fundamental tissue (partitions or sutures), and a white inner layer is nongelatinous, of hyphae that intertwine and run parallel with the surface of the gleba; the latter ochre to brown, becoming pulverulent at maturity; pseudocapillitium various lengths, $2.5-4\mu$ in diameter, much branched, hyaline, sparsely warty-spinulose; basidia forming a palisade-like hymenium on the walls of lacunae through which many hyphae penetrate, 4-spored; sterigmata short; spores spherical, echinulate, dark brown, $5-6\mu$ in diameter.

Type species: *Tremellogaster surinamensis* Ed. Fischer.

Habitat: On moist sandy soil, near decaying wood.

Distribution: British and Dutch Guiana, South America.

There is but the one species in the genus, and the above description serves very well for the species.

2. CLATHROGASTER Petri, *Malpighia* 14: 125-126. 1900. Description emended.

Fructifications hypogeous, subglobose, radicate, rhizomorph attached in a depression in the base; peridium thick, of 2 layers, outer of fundamental, periclinal hyphae, thin, silky, reticulately furrowed; inner layer thick, gelatinous, interrupted by more or less radial sutures of tissue like the outer peridium and connecting the latter with the gleba; lactiferous ducts large, long, penetrating all sterile tissues; gleba chambered, spongy, yellowish, cavities spherical to irregular, larger toward the outside, with one to many very large radiating, gelatinous, sterile cavities; septa broad, with stipitate basidia and cystidia on both sides; columella or prominent tramal plates radiating from the base or from a pulvinate sterile base; basidia mostly 2-spored; spores spherical, yellowish, reticulate with interrupted ridges, pedicellate.

The type species: *Clathrogaster volvarius* Petri.

Distribution: Sarawak, near Sibu, Borneo.

a. *Clathrogaster volvarius* Petri, *Malpighia* 14: 126. pl. 2, f. 1-2; pl. 3, f. 2, 3, 5-8, 10, 13; pl. 4, f. 1. 1900. (*Arcangeliella volvaria* (Petri) Z. & D. Mo. Bot. Gard. Ann. 22: 369. 1935; 23: 629. 1936.)

Fructifications irregular reniform about 4×6 cm., russet (in alcohol, 1934), surface irregularly reticulate-sulcate; sterile base scarcely more than a thickening of the peridium; columella conspicuous, branching near the base but branches percurrent or nearly so, gelatinous with many lactiferous ducts; peridium 1200–1440 μ thick (in alcohol), duplex, the outer layer (rind) of densely tangled hyphae, tough, somewhat gelatinous, the inner a thick gelatinous layer (tramal peridium), with many large lactiferous ducts, interrupted by radial plates (sutures) of tissue like the rind which connects the outer peridium and the *gleba*; the latter ochraceous-tawny, cavities ovoid, radiating from the columella and base; septa thick, of loose, gelatinous hyphae, with lactiferous ducts; basidia subcylindrical, 37–40 μ long, upper part collapsing after the separation of the spores; sterigmata short; spores spherical with short ridges and slender, blunt spines, yellowish, 9–12 μ in diameter.

Type locality: Sarawak, Borneo.

Distribution: Known from type locality only.

In his formal description of the species *C. volvarius*, Petri used the old spelling "*vulvarius*," but in all other cases throughout his paper he used "*volvarius*." Undoubtedly the use of the "u" in the one case was unintentional and thus our correction.

Petri did not designate a type species of *Clathrogaster*. *C. volvarius* Petri was chosen as the type since it was the better described and illustrated of the two species.

b. *Clathrogaster Beccarii* Petri, Malpighia 14: 126. pl. 2, f. 3–5, 7–9. 1900. (ut *C. Beccarii*.) (*Arcangeliella Beccarii* [Petri] Z. & D. Mo. Bot. Gard. Ann. 22: 366. 1935; 23: 635–636. 1936.)

Fructifications spherical to reniform, 1–3 cm. in diameter, raw sienna in alcohol, surface smooth but with low reticulated ridges; sterile base and columella not perceptible; peridium about 600–700 μ thick (in alcohol), duplex, the outer layer of compactly interwoven hyphae producing a rind, the inner layer thick, gelatinous of loosely woven hyphae mixed with lactiferous ducts, interrupted by radial plates (sutures) of fundamental tissue like the rind, and uniting the latter with the *gleba*; the latter amber-brown, cavities elongate, radiating from the base which is scarcely more than a thickened peridium; septa about 110 μ thick, similar to the inner peridium in structure; basidia clavate, 2-spored, about $80 \times$

11 μ , only the outer half collapsing after the separation of the spores; sterigmata short; spores 11–15 μ in diameter, spherical, with very high ridges, irregularly disposed over the surface, yellow.

Type locality: Sarawak, Borneo.

Distribution: Known from the type locality only.

B. GASTROSPORIACEAE Pilát, Bull. Soc. Myc. France 50: 45–46. 1934.

Description emended: Fructifications globose, hypogeous; peridium duplex; exoperidium of filamentous, fundamental tissues; endoperidium a continuous gelatinous layer, *not* interrupted by plates of fundamental tissue; gleba developing centripetally from anastomosing lamellae, produced subperidially, pulverulent at maturity; columella present; capillitium rudimentary; hymenium lining walls of cavities; spores spherical, minutely verrucose.

There is one genus, *Gastrosporium*.

1. GASTROSPORIUM Mattiolo, Memoria Accad. Sci. Torino, Ser. II 53: 361. 1903. (*Leucorhizon* Velenovsky in Mykologie 2 (3–4): 49–51. f. 1–4. 1925.)

Description emended: Fructifications hypogeous, globose; surface soft, white, dry; peridium duplex; outer layer filamentous, easily separable; inner layer tough, gelatinous, cartilaginous, indurated, continuous, easily distinguished from the gleba; the latter filling the whole peridium (no sterile base), white, becoming ochraceous to subolivaceous, with main tramal plates extending from a columella which is simple but reaching beyond the center of the fructification; hymenium lining cavities at first, but the whole pulverulent at maturity; capillitium rudimentary; spores light ochraceous, spherical, slightly verrucose.

Type species: *Gastrosporium simplex* Mattiolo.

Habitat: In the soil around the roots of grasses and sedges.

Distribution: Northern Italy and Czechoslovakia.

There is but one species in the genus *Gastrosporium*. Velenovsky, however, described a species *Leucorhizon nidificum* which becomes synonymous with *G. simplex*. It is peculiar that Velenovsky, Pilát¹⁴ and Mattiolo overlooked the fine verrucosity of

¹⁴ Pilát, A. Sur le genre *Gastrosporium* Mattiolo. Bull. Soc. Myc. France 50: 37–49. illus. 1934.

the spores. A collection under the name *Leucorhizon nidificum* Vel. from Bohemia is to be found in the Lloyd collections (Smithsonian Inst. Cat. No. 148).

GAUTIERIALES n. ordo

Fructificationes hypogaeae, sessiles, basi rhizomorphico instructae; peridio deficienti vel hyphis laxe intricatis vel pseudoparenchymatibus composito; gleba primo albida translucendo-cartilaginea, dein brunneola cartilaginea; columella simplici vel dendroidea, cartilaginea; septis hyphis gelificatis compositis, hymenophori; basidiosporis crasse fusiformibus, longitudinaliter costatis, brunneis.

Fructifications hypogeous, sessile; peridium usually wanting, when present stüpose, loosely filamentous, or pseudoparenchymatous; gleba gristly-translucent, whitish, becoming brownish as spores mature, with a columella from a basal rhizomorph; basidia in a hymenium; septa usually gelatinous-cartilaginous, of gelified hyphae; basidiospores of various shapes, mostly broad fusiform, longitudinally costate, brown.

Gautieriaceae n. fam. Characteribus ordinis.

The order Gautieriales, represented by the one genus *Gautieria*, partakes of the characters of several other groups of gasteromycetes but differs fundamentally from each enough that it cannot logically be classified with any. The fructifications follow the centrifugal, coralloid pattern of development and the gleba has a conspicuously gelatinous-cartilaginous consistency such as found in the Hysterangiales, but the gleba and spores do not partake of the phalloid nature of those of the latter. Nor does the peridium when present in species of *Gautieria* have the gelatinous or cartilaginous layers found in the same parts of the Hysterangiales. The spore type in the Gautieriales is similar to that found in *Hymenogaster*, *Gymnoglossum*, and *Secotium*, but the development of the fructifications is distinct as is the nature of the sterile tissues. The order Gautieriales differs from the Tremellogastrales in four fundamental respects. In the latter the gleba develops centripetally (lacunar), the peridium is characterized by gelatinous layers, the spores are spherical, and echinulate or verrucose, and the gleba becomes powdery at maturity.

To obtain the best concept of the genus *Gautieria* it is necessary to know several of its species as they occur in the field, so to

speak. Otherwise one does not become aware of the tough, gristly nature of the sterile tissues in most of the species. For instance, the type species *G. morchelliformis* Vitt. has such large glebal cavities and thin tramal septa that, although it has the same characters otherwise as the other species, the observer is not so impressed with its gelatinous-cartilaginous nature, as when he sees some other species of the genus with thicker septa, columella, etc. In fact the examination of dried specimens of *G. morchelliformis* may not reveal the real cartilaginous character of its tissues. In other words the type species of *Gautieria* does not represent the norm of the genus, but may be said to be almost marginal.

1. GAUTIERIA Vittadini, Monogr. Tuberac. 25-27. 1831.—not *Gautiera* Rafinesque Med. Fl. 1: 202. 1828. (Syn. *Chamonixia* Rolland, Soc. Myc. France Bul. 15: 76-77. 1899.)

Description emended: Fructifications subspherical to irregularly depressed, with a simple or branched rhizomorph sometimes persisting as a short stipe; columella variable, simple or branched, gelatinous to translucent-cartilaginous; peridium wanting, evanescent, or persistent, when early evanescent or wanting outer septa sterile over the surface; gleba white or gristly-translucent, becoming brownish as spores mature; cavities labyrinthiform; septa usually thick, gelatinous-cartilaginous, of interwoven, gelatinized hyphae; basidia in hymenium covering both sides of septa; spores ovoid, ellipsoid, fusiform, with longitudinal striae, brown.

Type species: *Gautieria morchelliformis* Vitt.

Habitat: Wholly or partially hypogeous under various kinds of shrubs and trees.

Distribution: Europe, Asia, Africa, North and South America, Australia, Tasmania, New Zealand.

The species of *Gautieria* have been published elsewhere.¹⁵

Eleven species have been reported from North America and eight others occur elsewhere in the world.

A NEW FORM ORDER OF FUNGI IMPERFECTI

Lycoperdellon Torrend (in Broteria Sér. Bot. 11: 92. 1913) has had a very doubtful taxonomic position. Fischer (1933, p.

¹⁵ Dodge, C. W., and S. M. Zeller, Mo. Bot. Gard. Ann. 21: 692-705. pl. 18, f. 51-66. 1934.

72) referred it to the doubtful list of genera under the *Lycoperdaceae*. To be sure, the fructifications of *Lycoperdellon* have the general appearance of a myxomycete, like *Lycogala*, or a lycoperdaceous gasteromycete but there are no basidia or basidiospores. Lohwag¹⁶ accordingly recognized the sporophores as real conidiophores and thought them to represent the conidial stage of an ascomycete. The conidial stages of ascomycetes so far as we know, however, do not take on the form of a closed sporocarp similar to a gasteromycete. There is no doubt *Lycoperdellon* is one of the Fungi Imperfecti, but its closest relatives are doubtless among such forms as *Leucophleps* Harkness, all the species of which, except *L. candida* Harkness, have proved to be the conidial stage of one or another of the species of the gasteromycetous genus, *Leucogaster* Hesse. After considerable careful study of *Lycoperdellon* by Heim and Malençon¹⁷ who continued to designate its spores as "conidia," Heim described the family *Lycoperdellaceae* to receive the genus *Lycoperdellon* with its two species *L. Torrendii* (Bresad.) Torrend and *L. minutum* Heim.¹⁸ Unfortunately he assigned the family to the Gasteromycetes whereas from our viewpoint it belongs in the Imperfecti. It is, therefore, proposed to transfer the family *Lycoperdellaceae* Heim to the Fungi Imperfecti and refer to it the form genera *Lycoperdellon* Torrend and *Leucophleps* Harkness. There will need also to be the Form Order *Lycoperdellales*¹⁹ coordinate with the Phyllostictales, Melanconiales, and Moniliales. In a key like Martin's²⁰ the new form order would key out as follows: "Fructification determinate, gasteromycetoid; conidia borne in chambered cavities or nests."

Leucophleps candida Harkness has been found in western Oregon and central California. Neither species of *Lycoperdellon* has been reported from North America.

¹⁶ Lohwag, H. Zu *Lycoperdellon*. Ann. Myc. 32: 244-255. 1934.

¹⁷ Heim, Roger, and G. Malençon. Le genre *Lycoperdellon*: structure et position taxonomique. Rev. Gen. de Bot. 45: 53-67. 1933.

¹⁸ Heim, R. Fungi Ibirici. Treballs del Mus. Cien. Nat. Barcelona 15 (No. 3): 1-146. 1934. (See pp. 138-141.)

¹⁹ Fructificationes gasteromycetoides, sed conidia gignentes.

²⁰ Martin, G. W. Key to the families of fungi. Univ. Iowa Stud. in Nat. Hist. 17: 83-115. 1936. (See p. 90.)

STUDIES IN THE DARK-SPORED AGARICS *

ALEXANDER H. SMITH

(WITH 93 FIGURES)

The present study deals chiefly with notes on the microscopic characters of the types of some of the species of *Coprinus*, *Naematoloma*, *Panaeolus* and *Psilocybe* described from North America. As has long been recognized by investigators who are working in this group, the descriptive accounts, whether old or new, which do not place great emphasis on spore morphology, on the characters of the cystidia if such are present, and on the anatomical features of the fruiting bodies, are of little value in accurately delimiting taxonomic entities of any category. It naturally follows, then, that one of the first steps in a critical revision of these genera is to ascertain the diagnostic characters from the type specimens in so far as the latter are available. However, as I found out early in my work in this group, an extensive knowledge of these fungi as they occur in nature is an invaluable aid in interpreting the characters of dried specimens. Consequently, although my investigations in the group have been in progress for over ten years, it is only comparatively recently that I have established species concepts in many of the genera that are satisfactory to me, and which serve satisfactorily as the building units for a classification which is other than just haphazard.

Considerable progress has been made in the recognition of species in the field through the process of mass collections, *i.e.*, collecting material of *all* fruitings of dark-spored agarics found during a season. Through such methods one soon learns to recognize the frequently-encountered species and to ascertain the constancy of their characters even though he may be unable to apply binomials correctly to them. The studies of type specimens do much

* Papers from the Herbarium and the Department of Botany of the University of Michigan. The cost of the illustrations was paid for by the University of Michigan Herbarium.

to clear up the application of names, and the final result should be a satisfactory classification in which the species are adequately described. This, at least, is the aim in the manuscript being prepared for the *North American Flora*.

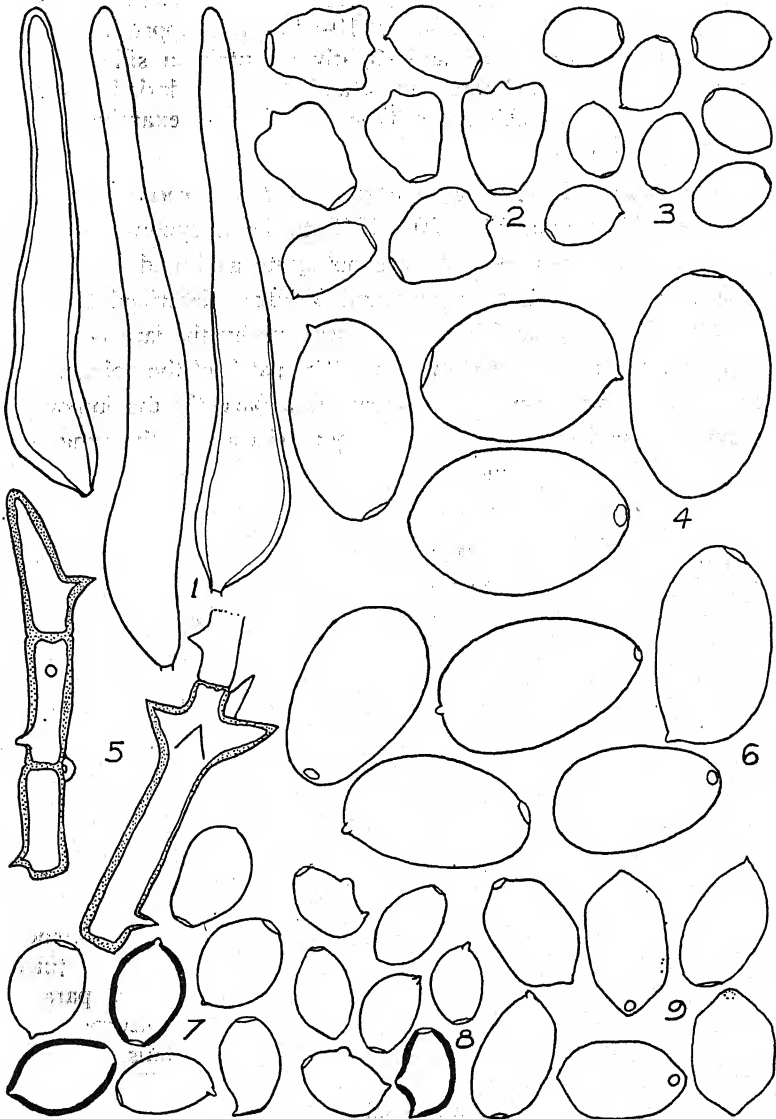
Because of the interest being shown in dark spored agarics both by European and American workers at the present time, and because of the general impetus given to the study of fleshy fungi by those seeking new antibiotics for medicinal use, it is urgent to make available such data as that presented here. Since the characters of the spores are fundamental, and since they have been badly handled by past investigators, detailed descriptions as well as illustrations are included. Although the genera treated here are classed as "dark-spored" fungi, it must be remembered that in *Stropharia*, *Naematoloma* and *Psilocybe* this character breaks down, and species with earth-brown to cinnamon-brown spore deposits must be admitted if any emphasis at all is placed on obvious natural relationships. The more material I examine, the more I become convinced that actually the genus *Agrocybe* of modern authors should be placed here also.

For access to type specimens reported on here I am indebted to Dr. H. D. House, State Botanist, New York State Museum, Albany, New York, to Dr. Fred J. Seaver, of the New York Botanical Garden and to Dr. Rolf Singer, Farlow Herbarium, Harvard University, Cambridge, Mass.

COPRINUS

COPRINUS ANGULATUS Peck, Ann. Rep. N. Y. State Mus. 26: 60. 1874. (FIGS. 1-2-*type*.) Spores 8.5-10 (11) \times 5.7-6.3 \times 7-8.8 μ , dark bistre to blackish revived in KOH, somewhat flattened, truncate-ellipsoid in side view, resembling a blunt arrow head in face view, apical hyaline pore broad and conspicuous; basidia 18-32 \times 7-9 μ , four-spored, trimorphic; paraphyses inflated, hyaline, readily collapsing; pleurocystidia voluminous, ellipsoid to cylindric (50) 60-100 (120) \times (15) 20-60 μ , hyaline, smooth, thin-walled and readily collapsing; cheilocystidia vesiculose and 15-40 μ in diam., or fusoid-ventricose [the latter 38-46 (55) \times (7) 9-14 μ], both types thin-walled and hyaline; gill trama hyaline in KOH or colored cinnamon-brown toward pileus trama; pileus trama with a cuticle of vesiculose cells one cell deep, from

among them arise numerous pilocystidia (32) $60-90 \times 9-16 \mu$, subcylindric to ventricose at base, apices obtuse to subacute, the walls thin and hyaline or slightly thickened in basal part and either hyaline or tawny as revived in KOH; flesh beneath cuticle tawny to cinnamon-brown in KOH.



FIGS. 1-9. Microscopic characters of dark-spored agarics.

Discussion. Peck's original description and comments indicate that this species belongs in the *C. ephemerus* group, and the present study of the type makes this disposition unquestionable. The pilocystidia are abundant and unmistakable.

COPRINUS CALYPTRATUS Peck, Bull. Torr. Club 22: 205. 1895. (FIG. 4-type.) Spores $17-21 \times 10-13 \mu$, not appreciably flattened, hyaline pore small and slightly eccentric in side view of spore, walls only moderately thick and in KOH dark bister (no other details obtainable from type, and spores examined were slightly immature).

Discussion. I consider *Coprinus asterophorus* Long and Mentzer, Mycologia 37: 120. 1945 to be a synonym of this species. Its spores (FIG. 6) are not quite as broad in the one collection illustrated, but Long and Mentzer described them as $14-20 \times 10-12.7 \mu$, and in material sent me by the late Dr. Long considerable variation was evident. The patch of the volva which forms a yellowish cap on the pileus is apparently the important diagnostic field character. In its spore characters the fungus is very similar to *Coprinus sterquilinus* but differs sharply in the more compact organization of the veil tissue.

COPRINUS BRASSICAE Peck, Ann. Rep. N. Y. State Mus. 43: 18. 1890. (FIG. 3-type.) Spores $6.2-7.8 \times 3.6-4 \mu$, dull cocoa brown when first revived in KOH, not flattened, ellipsoid in either view, hyaline apical pore distinct but small; basidia four-spored, hyaline in KOH; (no other microscopic characters determinable on material examined).

Discussion. Unfortunately the characters of the veil could not be determined. These are important in this group. The species has received considerable attention in the American literature.

COPRINUS CINCHONENSIS Murrill, Mycologia 10: 85. 1918. (FIGS. 5 & 7-type.) Spores $9-11 \times 5-6 \times 6-7.5 \mu$, subelliptic in side view, ovoid in face view, terete or only slightly flattened, smooth, pale bister in KOH, apical pore hyaline and distinct; basidia hyaline in KOH, four-spored, $7-8 \mu$ broad at apex; paraphyses hyaline, thin-walled, inflated, $10-14 \mu$ diam.; pleurocystidia apparently present but remaining collapsed and details not clear, hyaline and thin-walled; cheilocystidia none seen; gill and pileus trama hyaline in KOH; universal veil remnants in the form of matted fibrils in patches over the disc (no globose cells seen),

the hyphae much-branched, with somewhat thickened walls and with scattered short thorn-like processes unevenly distributed over them, the cells nearly hyaline to slightly yellowish in KOH.

Discussion. This is a distinctive species if one considers the characters of the veil and spores in conjunction with the habitat on a log. It appears to be closely related to *Coprinus Brassicae* Peck but is readily distinguished on spore size.

COPRINUS EBULBOSUS Peck, Bull. Torrey Club 22: 491. 1895. (FIG. 8—*type*.) Spores $7-8.4 \times 4.4-5 \mu$, dull chocolate-brown revived in KOH, smooth but often with a ventral hump as seen in side view (apparently a second pore), oblong to subellipsoid in face view, subellipsoid to slightly inequilateral in side view, apical pore present but obscure; basidia four-spored, hyaline in KOH, $18-24 \times 7-8 \mu$, apparently trimorphic; paraphyses $10-16 \times 10-16 \mu$, subglobose, hyaline in KOH; pleurocystidia scattered; subcylindric, $80-120 \times 18-32 \mu$, thin-walled and readily collapsing; cheilocystidia none seen; gill trama of filamentous hyphae, hyaline in KOH; pileus trama hyaline in KOH, cuticle of vesiculose hyaline cells about 1 cell deep.

Discussion. Both the collection from Lyndonville, N. Y. and the one from Kansas show the curious ventral hump on at least some spores so there is no doubt but that both represent the same species. It is very closely related to *C. quadrifidus* Pk., but apparently differs in slightly narrower spores. However, this difference is not truly significant. From the information at hand it is not clear whether the ventral hump or second pore is an abnormality which occasionally occurs in the members of the *quadrifidus* group and is comparable to the bifid spores as reported by Lange (p. 743) for *C. myceliocephalus*, or whether it is a useful character.

COPRINUS HEXAGONOSPORUS Jossierand, Bull. Soc. Myc. Fr. in press. (FIG. 9.) Pileus cylindric at first, 5-10 (15) mm. high, 4-6 (8) mm. across the base, very finely pubescent at least over the disc, disc "fuscous," marginal area pallid to "avellaneous," tinged "army brown" near the disc at maturity, surface closely folded-striate before expanding, becoming broadly convex to plane and plicate-striate before maturity; flesh very delicate and thin, odor none, taste mild to slightly bitterish; lamellae free but attached to apex of stipe, close, whitish, soon avellaneous and then black, narrow and equal (1-2 mm. broad), soon deliquescing; stipe 3-5 (10) cm. long, 0.5-1 (2.5) mm. thick, equal, hollow,

very fragile, sparingly pubescent at first from projecting caulocystidia, densely pubescent above, white at first but soon sordid brownish over lower two thirds, base inserted on the substratum.

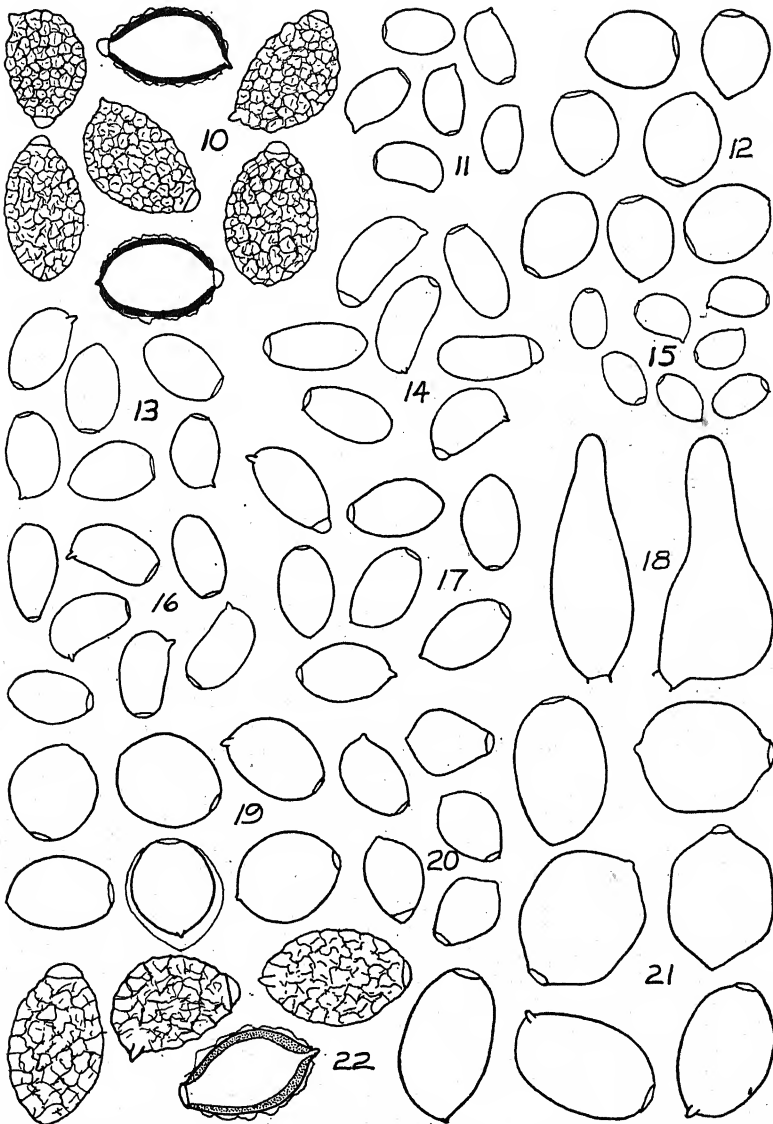
Spores black in deposit, $11-13 \times 5-6.2 \times 6.5-8 \mu$, black in KOH, flattened, subelliptic in side view, angular and obscurely six sided in face view, pore eccentric as seen in side view; basidia trimorphic, $18-26 \times 8-10 \mu$; paraphyses $18-22 \times 9-16 \mu$; pleurocystidia rare, $60-90 \times 20-35 \mu$, becoming more elongated and narrower, readily collapsing; cheilocystidia similar to pleurocystidia or more vesiculose, $40-80 \times 35-45 \mu$; gill trama narrow and of very loosely interwoven cells; pileus trama with a cuticle formed by a row of vesiculose cells from which thin-walled pilocystidia project, the latter $70-120 \times 10-15 \mu$, trama proper very thin but hyphae filamentous, those in the dark discal area dark sordid brown (almost vinaceous brown) when revived in KOH.

Discussion. This fungus, a close relative of *C. ephemerus*, is apparently quite common on horse dung in the United States. I have isolated it on numerous occasions. Brooks obtained it from horse dung collected in Kansas, and both Brooks and Lange isolated it from the same substratum collected near Ann Arbor. The spores in face view are the outstanding feature of the species, but once the fungus is known it can usually be identified at sight. It has not been previously reported from North America.

COPRINUS INSIGNIS Peck, Ann. Rept. N. Y. State Mus. 26: 60. 1874. (FIG. 10-type.) Spores $10-12.6 \times 7-8.4 \mu$, dark bistre in KOH, the exospore wrinkled and cracked to form obscure warts or causing surface to appear decidedly uneven, in side view slightly inequilateral, in face view oblong or base somewhat pointed, apex with a protruding lens-shaped hyaline pore causing it to appear snout-like; basidia four-spored, hyaline in KOH, paraphyses inflated, readily collapsing; pleurocystidia apparently subcylindric, elongated and projecting across the gill cavity (but remaining collapsed); cheilocystidia none seen; gill and pileus trama hyaline in KOH, the latter homogeneous or nearly so (with no sharply defined cuticle in sections of revived material).

Discussion. This well-known and characteristic species of the *C. atramentarius* group is known from both North America and Europe. The occurrence in several sections of *Coprinus* of rough spored and smooth-spored species with almost identical macroscopic characters is interesting in the light of recent efforts to

place great emphasis on spore markings in determining natural relationships among the fleshy fungi. The markings on the spores of *Coprinus* form excellent characters for the recognition of species, but if a natural arrangement is desired, it would be a serious



FIGS. 10-22. Microscopic characters of dark-spored agarics.

mistake to insist that all species with rough spores were more closely related to each other than to any with smooth spores. It is clearer in *Coprinus* than in almost any other genus that the character of ornamented spores has evolved independently on numerous occasions.

COPRINUS JALOPENSIS Murrill, *Mycologia* 10: 83. 1918. (FIG. 11-type.) Spores $6-6.3 \times 3.1-3.6 \mu$, cocoa-color as revived in KOH but gradually becoming grayish, smooth, not flattened, slightly bean-shaped in side view, subelliptic in face view, smooth, apical pore hyaline and inconspicuous; basidia hyaline in KOH, $12-18 \times 5-5.5 \mu$, four-spored, trimorphic; paraphyses thin-walled, hyaline, readily collapsing, $9-12 \times 6-9$ (12) μ , enlarging to greatest size at time spores are discharged; pleurocystidia abundant and voluminous, hyaline, thin-walled and readily collapsing, extending across the gill cavity and $20-40 \mu$ in diam.; cheilocystidia not reviving, the gill edges whitish apparently from filamentous hyphae; gill trama not reviving well but hyaline in KOH; pileus trama tawny yellowish to tawny-orange just under the cuticle, cuticle of a single layer of inflated cells, no pilocystidia or universal veil remnants found.

Discussion. This species must belong in the *C. radians* group, though its veil characters, if a veil is present, are not known. The oozonium and the small cocoa-colored spores along with the lignicolous habitat are certainly suggestive. The very small spores appear to characterize it in this group.

COPRINUS JONESII Peck, *Bull. Torrey Club* 22: 205. 1895. (FIG. 12-type.) Spores $7-9 \times 6-7 \mu$, terete to very slightly compressed, subellipsoid in side view, subcircular in face view, black or nearly so in KOH, exospore separable from endospore only under considerable pressure (enough to break inner spore wall in many instances), apical hyaline pore broad and distinct; basidia four-spored, hyaline in KOH, $7-8 \mu$ in diam., trimorphic; paraphyses hyaline, thin-walled, readily collapsing, $10-12 \mu$ broad; pleurocystidia projecting across gill cavity but not reviving well, apparently long-cylindric and thin-walled; cheilocystidia none seen; gill and pileus trama hyaline in KOH; universal veil remnants filamentous, hyaline to sordid brownish in KOH.

Discussion. A member of the *C. lagopus* series, but distinct on spore characters. *C. lagopus* var. *rotundisporus* Kuhner & Josseland is either identical with or very close to Peck's species.

COPRINUS LANIGER Peck, Bull. Torrey Club 22: 491. 1895. (FIG. 14-type.) Spores dark sordid reddish brown when first revived in KOH but soon chocolate-grayish, $8-10.5 \times 3.5-4.2 \mu$, smooth, not flattened, narrowly ellipsoid to subcylindric in face view, in side view almost straight to decidedly concave on inner side and the back distinctly to merely obscurely convex, lens-shaped pore apical and conspicuous when not collapsed; basidia four-spored, $14-24 \times 5-6 \mu$, trimorphic, hyaline in KOH; paraphyses inflated, $12-15 \times 8-12 \mu$, hyaline, readily collapsing; pleurocystidia scattered, ellipsoid to subcylindric, $50-90$ (or more) $\times 16-30 \mu$, thin-walled and readily collapsing; cheilocystidia not seen (cells had deliquesced); gill trama hyaline to faintly yellowish in KOH; pileus trama merely yellowish in KOH, the cuticle of inflated cells one cell deep; universal veil remnants distinctive: the layer next to cap surface of chains of globose to keg-shaped hyaline cells, the cells of the chains more elongated outward as well as more brownish and the filaments finally composed of more or less ellipsoid to cylindric, tawny-cinnamon (in KOH), somewhat thick-walled cells which are smooth except for obscure zones and lines of encrusting pigment, the end cell often somewhat fusoid and cystidium-like, the chains of cells showing a tendency to break up into short segments or individual cells.

Discussion. This species is very closely related to *C. domesticus*, but differs from it in the long narrow spores with the very broad germ pore. The clustered habit of growth is a less constant distinguishing character. It is one of the extreme variants of the *domesticus*-*radians* series which at least for the present is being recognized as a species.

COPRINUS MEXICANUS Murrill, Mycologia 10: 84. 1918. (FIG. 15-type.) Spores $4.7-5.3$ (6) $\times 3.1-3.6 \mu$, pale cocoa-brown but soon changing to pale avellaneous when revived in KOH, many nearly hyaline, smooth, not flattened, ellipsoid to slightly inequilateral in side view, ellipsoid in face view, apical hyaline pore very small and inconspicuous; basidia and paraphyses not reviving; some fusoid-ventricose cystidia seen in crushed mounts of gills, these $28-36 \times 9-12 \mu$ and with obtuse to subacute apices; gill trama not reviving; pileus trama with a cuticle of narrow ($3-5 \mu$) radially arranged hyphae heavily encrusted with a cinnamon-brown pigment (revived in KOH), beneath this occur scattered enlarged hyphae interwoven with typical filamentous strands but no true hypoderm differentiated; flesh proper hyaline in KOH; universal veil remnants hyaline to sordid yellowish in KOH, filamentous.

Discussion. This is a most unusual species with a veil like that of *C. myceliocephalus* M. Lange (p. 742), but apparently not closely related to it. It very likely belongs in the *C. phaeosporus* group though the fibrillose cuticle is unusual even there. Its spores are among the smallest I have seen in the genus.

COPRINUS PSEUDORADIATUS Kühner and Josserand, Bull. Soc. Myc. Fr. 60: 26. 1944. (FIG. 13.) Pileus 3–5 mm. high, 3–4 mm. broad when expanded, expanding to plane and 5–7 mm. broad, at first completely covered by a dense hairy-fibrillose universal veil which breaks up into white squarrose squamules and soon disappears, surface grayish white before spores mature, soon becoming lead gray to blackish, wrinkled-striate to the smooth disc, soon splitting radially; flesh very delicate; lamellae free, white, becoming jet black before deliquescing, close, edges white-fimbriate; stipe 2–3 cm. long, 0.5 mm. in diam., equal, white at first, the lower part with recurved fibrillose squamules from the remains of the veil, glabrescent, base slightly tomentose.

Spores black in deposit, $7-8.4 \times 4-4.5 \mu$, blackish revived in KOH, ellipsoid, not flattened, apical hyaline pore small and inconspicuous; basidia four-spored; pleurocystidia and cheilocystidia similar, $40-60 \times 10-18 \mu$, ellipsoid to cylindric; fibrils of the veil of long-cylindric cells $60-90 \times 12-18 \mu$, next to the cap surface the cells often short and ovoid.

Discussion. I first collected this fungus in Nova Scotia, July 27, 1931, in Colchester County. However, only a few caps were collected and a description was withheld pending the collection of more and better specimens. In the meantime Kühner and Josserand discovered it in France. During the summer of 1946 it was collected in the vicinity of the University of Michigan's Biological Station at Douglas Lake. The North American collections have all been from rabbit dung.

COPRINUS PULCHRIFOLIUS Peck, Ann. Rept. N. Y. State Mus. 29: 41. 1878. (FIG. 16-type.) Spores $7-8.4 \times 4-4.2 \mu$, reddish brown, becoming gray in KOH, ellipsoid in face view, slightly curved in side view or merely straight on ventral line and convex on dorsal line, apical hyaline pore distinct; basidia $6-7 \mu$ in diam., four-spored, hyaline in KOH; paraphyses inflated and hyaline in KOH; pleurocystidia none found (careful search made); cheilocystidia none seen (gill edges had apparently deliquesced); gill trama hyaline in KOH; pileus trama hyaline in KOH or slightly yellowish where not revived well, cuticle of globose, hyaline, in-

flated cells; universal veil remnants also of hyaline, more or less globose-inflated cells readily separable from each other.

Discussion. This is one of the *Coprinus* closely related to *C. radians*. A final disposition of it must await a critical study of the group based on numerous collections. I have been able to recognize both species in the vicinity of Ann Arbor.

COPRINUS QUADRIFIDUS Peck, Ann. Rept. N. Y. State Mus. 50: 106. 1897. (FIG. 17—*type*.) Spores smooth, dark chocolate color revived in KOH, $7.5-9.5$ (10.5) \times $4-4.5$ (5.5) μ , very slightly compressed to terete, ellipsoid in side view, slightly ovoid in face view, apex truncate from a hyaline pore; basidia four-spored (12) $14-16$ (18) \times $6.5-7.5$ μ , hyaline, trimorphic; paraphyses $9-11 \times 8-10$ μ , vesiculose, hyaline, readily collapsing; pleurocystidia abundant, subcylindric, $100-150 \times 20-35$ μ , hyaline, thin-walled, readily collapsing, at times extending across the gill cavity; cheilocystidia present at first and $50-80 \times 15-25$ μ , subellipsoid, soon collapsing; gill trama hyaline in KOH; pileus trama hyaline, cuticle of a layer of hyaline vesiculose cells several cells deep, veil remnants of filamentous hyphae $6-10$ μ in diam., clamp connections present.

Discussion. This is one of our best known American species. In none of my collections have spores with a ventral hump or pore been present, and the species is common locally in June. In *C. ebullbosus* this character is quite pronounced. Aside from this one character both appear referable to a single species. The situation here parallels that found in *C. atramentarius*. See *C. variegatus* for further comments.

COPRINUS ROTUNDISPORUS Peck, Ann. Rept. N. Y. State Mus. 31: 35. 1879. (FIGS. 18-19—*type*.) Spores $8-10 \times 6-7 \times 7-9$ μ , black when first revived in KOH but the dark pigment of the exospore soluble in KOH and gradually dissolving in the mount to a bister solution leaving the spore with a dark bister endospore and a practically hyaline exospore, in side view subellipsoid, slightly flattened, in face view broadly ovate when first revived but when only endospore is colored appearing subcircular, apical hyaline pore broad and distinct, exospore separable from endospore if mounts in KOH are slightly crushed under a cover glass; basidia four-spored, $15-22 \times 5-6$ μ , hyaline in KOH, difficult to demonstrate and apparently either not projecting or only dimorphic; paraphyses greatly enlarged, $16-20 \times 10-18$ μ , globose

to ellipsoid, apparently more or less filled with a mucilaginous substance, not collapsing but instead reviving exceptionally well in KOH and very distinct by the manner in which the transmitted light is refracted; pleurocystidia scattered, not coprinoid but instead typically fusoid-ventricose as in most agarics and highly refractive like the paraphyses, $28-36 \times 9-14 \mu$, apices obtuse; cheilocystidia none seen; gill trama hyaline in KOH and reviving well, highly refractive, with a narrow central strand consisting of a few filamentous hyphae, these flanked on either side by subglobular to elliptic highly refractive cells resembling the paraphyses, cap obviously splitting down the backs of the gills along the layer formed by the filamentous hyphae; pileus trama mostly of highly refractive, large, well-revived hyaline (in KOH) cells, cuticle of narrow ($4-7 \mu$) radially arranged hyphae (which may possibly have been veil remnants) also highly refractive and hyaline in KOH; veil remnants filamentous and not sharply distinguishable from those forming the apparent cuticle; no clamp connections seen.

Discussion. This fungus has so many curious microscopic characters for a *Coprinus* that it should be easy to recognize it in spite of the lack of sufficient data on the macroscopic features. The behavior of the pigment in the exospore is unique, the type of pleurocystidium very unusual, and the refractive nature of the paraphyses anomalous.

COPRINUS SEMILANATUS Peck, Ann. Rept. N. Y. State Mus. 24: 71. 1872. (FIG. 21-type.) Spores $12.5-15 \times 7-8.4 \times 9-12.5 \mu$, nearly coal black as revived in KOH, flattened, subellipsoid in side view, obscurely angular and broadly elliptic to subcircular in outline in face view, hyaline pore apical and inconspicuous or projecting slightly to form a snoutlike apex; details of gill trama and hymenium not obtainable; pileus trama hyaline in KOH; universal veil remnants of thin-walled readily collapsing globose to barrel-shaped cells.

Discussion. A member of the *Coprinus niveus* group, but a species rather easily distinguished in nature. Both the spore characters and the veil place it here.

COPRINUS SEYMOURII Peck, Ann. Rept. N. Y. State Mus. 28: 49. 1876. (FIG. 20-type.) Spores $6.2-7.8 \times 3.1-3.5 \times 4-4.6 \mu$, dull rusty bister in KOH, changing to chocolate-color, flattened, subellipsoid in side view, obscurely triangular in face view, hyaline

pore apical and distinct; basidia hyaline in KOH, four-spored, $5-6\mu$ in diam.; paraphyses hyaline, inflated, readily collapsing; pleurocystidia none seen (type had been pressed and gills did not revive well); cheilocystidia none seen; gill trama not reviving; pileus trama with a cuticle of vesiculose cells, the walls thickened in the angles at the base and brownish in KOH.

Discussion. A member of the *C. micaceus* series. I have a number of collections which have been referred here, but as yet am not satisfied that the species is more than an extreme variant of *C. micaceus*. If the habitat is truly terrestrial, that should be an aid in distinguishing it, but *C. micaceus* can be very deceiving in this regard.

COPRINUS SILVATICUS Peck, Ann. Rept. N. Y. State Mus. 24: 71. 1870. (FIG. 22-type.) Pileus 1-2 cm. high in buttons, 0.5-2.5 cm. across the base when expanding, ellipsoid to subovoid in buttons and then densely pubescent from cystidia, wrinkled-striate to disc but striae extending unequal distances, disc ochraceous tawny to tawny or this color over all when young, striate portion soon ochraceous buff to pale ochraceous buff and very atomate, disc glabrous at maturity, marginal area finally livid and splitting as the spores are shed; flesh very thin and fragile, no odor or taste; lamellae equal, attached at apex of stipe, moderately broad, close to crowded, pallid young, darkening over all before starting to deliquesce, edges white-fimbriate at first; stipe short, 1.5-2.5 cm. long, 1-2.5 mm. thick, equal, margin of cap attached at base but bulb if present soon evanescent (but expansion as in *Bulbopodium* of *Cortinarius*), densely pubescent at first, finally glabrous, pallid over all or at base slightly sordid.

Spores $11.5-14 \times 7-8.4 \times 8-9\mu$, slightly broader in face view than in side view (slightly compressed), slightly inequilateral in side view, subovoid in face view, apical pore broad, hyaline, prominent and causing apex to appear more or less snoutlike, surface slightly wrinkled from a thin hyaline somewhat deciduous exospore, dark bister to blackish revived in KOH; basidia four-spored, hyaline in KOH, trimorphic; paraphyses inflated, hyaline in KOH; pleurocystidia and cheilocystidia none seen (sections revived poorly); gill and pileus trama dark brown when first mounted in KOH but rapidly clearing to sordid yellowish, cuticle of pileus of vesiculose cells about one cell deep, numerous hyaline, thin-walled, subcylindric to basally ventricose pilocystidia $80-120 \times 10-18\mu$ arising from this layer, their necks greatly elongated and $10-15\mu$ in diam., their apices rounded to obtuse.

Discussion. The description of the macroscopic characters is taken from numerous collections made by Connors, Groves, and Smith in the vicinity of the laboratories at the Petawawa Experiment Station near Deep River, Ontario, during the season of 1947. In this material the basidia varied from two- to four-spored and the spores $14-20 \times 7.5-10 \mu$. The paragraph on the microscopic data given here was taken from the type. The species stands out as very distinct in the *C. ephemerus* series.

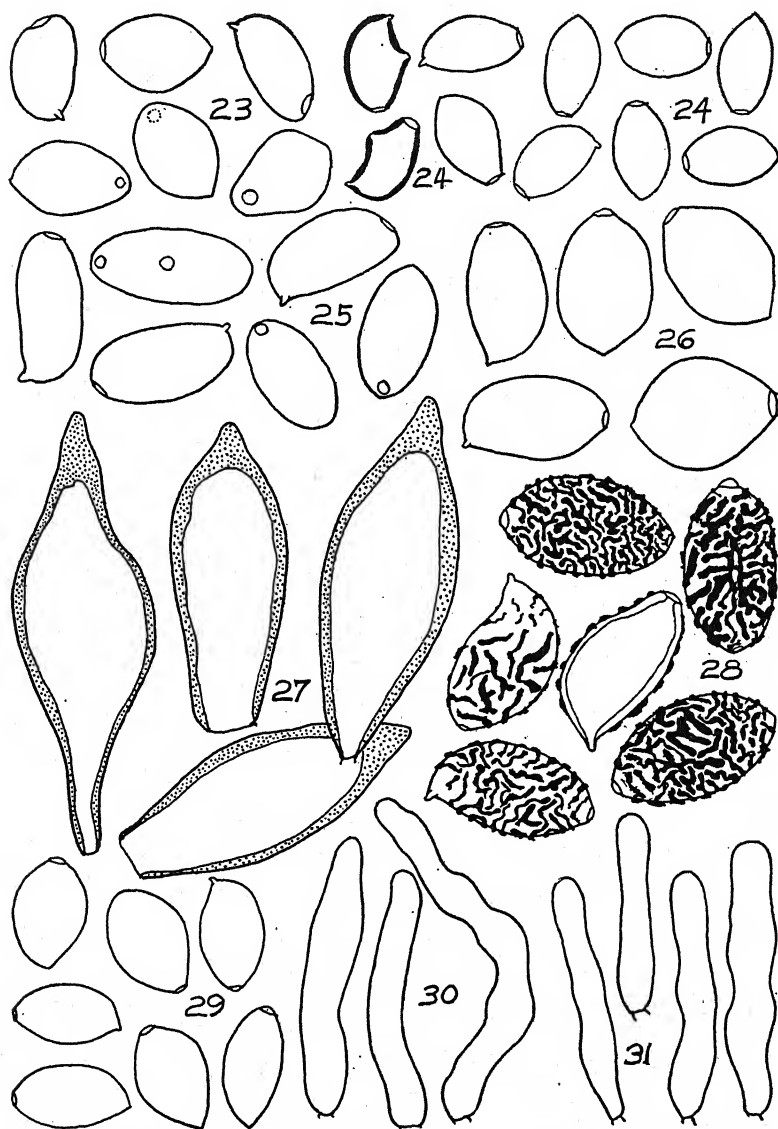
COPRINUS SPRAGUEI Berk. & Curt. Ann. and Mag. Nat. Hist. III 4: 292. 1859. (FIG. 23.) Spores bister in KOH, $9-11 \times 5.5-6.5 \times 6.5-7.8 \mu$, slightly flattened, suboblong in side view, in face view obscurely angular-ovate, base broadly pointed, the hyaline pore distinctly eccentric but small and inconspicuous; basidia hyaline in KOH, four-spored, $18-32 \times 6-7.5 \mu$; paraphyses $14-18 \times 9-13 \mu$, hyaline and inflated; pleurocystidia and cheilocystidia none seen; gill trama hyaline in KOH; pileus trama homogeneous beneath a cuticle formed by a palisade of more or less ellipsoid cells, flesh tawny in KOH just beneath the cuticle in thick sections (material revived poorly and KOH test not truly distinctive), no pilocystidia seen.

Discussion. The microscopic data are taken from a collection Murrill compared with the type at Kew and pronounced identical with it. The species is clearly in the *C. plicatilis* series and distinct because of its smaller spores.

COPRINUS VARIEGATUS Peck, Bull. Buffalo Soc. Nat. Sci. 1: 54. 1873. (FIG. 24-type.) Spores $7-9 (10) \times 5-6.2 \mu$, subellipsoid to ovoid, not flattened, often with a ventral hump or blister (germ pore?), dull cocoa-brown when first revived in KOH, gradually fading to near avellaneous or "wood brown" (possibly immature), apical hyaline pore distinct; basidia four-spored, hyaline in KOH, $14-22 \times 5-7 \mu$, clavate to subcylindric on a short pedicel, trimorphic; paraphyses hyaline, thin-walled, readily collapsing; pleurocystidia cylindric, hyaline, thin-walled, readily collapsing, $60-120 \times 15-28 \mu$; cheilocystidia none seen; gill trama hyaline in KOH; pileus trama hyaline in KOH, cuticle of narrow ($4-8 \mu$) somewhat gelatinous hyphae as revived in KOH.

Discussion. This species is apparently easily distinguished from *C. ebubosus* and *C. quadrifidus* by the filamentous somewhat gelatinous cuticle. However, further studies on either fresh or very carefully dried specimens of all three are needed to establish this

difference as a valid character. The general aspect of the fruiting bodies and the type of spore remind one of *C. ebulbosus* and *C. quadrifidus*. The white rhizomorphs and peronate-annulate stipe may be additional diagnostic characters.



FIGS. 23-31. Microscopic characters of dark-spored agarics.

Coprinus subpurpureus sp. nov. (FIG. 25—*type*.)

Pileus 1.5 cm. altus, demum 2–3.5 cm. latus, pubescens, purpureo-brunneus vel subpurpureus; lamellae anguste adnatae, confertae, pallidae demum nigrae; stipes 4–10 cm. longus, 1–2.5 mm. crassus, cavus fragilissimus, lilaceo-umbrinus demum pallidus, pubescens; sporae $12-14 \times 5.5-6.8 \times 7-8 \mu$.

Pileus 1.5 cm. high, up to 3.5 cm. broad when expanded, finely pruinose-pubescent when young, soon glabrous as cystidia collapse, "natal brown" to "Benzo brown" on disc, "light brownish drab" toward the paler ("light cinnamon drab") broad, striate, marginal area, in age "dark purple drab" over disc and dark gray to blackish over margin; flesh very thin and fragile, delicate, no odor or taste; lamellae narrowly adnate, close, becoming subdistant, narrow, near "tilleul buff," darkening to black over all before the edges deliquesce; stipe 4–10 cm. long, 1–2.5 mm. thick, equal, dull lilac umber young, pallid in age, densely pruinose-pubescent but soon glabrescent, base white-strigose.

Spores black in deposits, $12-14 \times 5.5-6.8 \times 7-8 \mu$, smooth, germ pore eccentric, in face view spores with a ventral pore visible (but not humped as in *C. ebulbosus*), narrowly subovate to elliptic in face view, with a suprahilar depression in side view; basidia four-spored, tetramorphic, $18-30 \times 6-7 \mu$; paraphyses hyaline, inflated, not otherwise distinctive; pleurocystidia none seen; cheilocystidia vesiculose to broadly fusoid-ventricose, $10-30 \mu$ in diam.; gill trama very thin and appearing cellular in sections, slightly colored vinaceous brown in KOH (young gills); pileus trama thin and appearing to be a mixture of narrow filaments and enlarged cells, cuticle a single layer of vesiculose to pedicellate cells from which thin-walled pilocystidia $60-100 \times 10-16 \mu$ project, layer beneath cuticle lilaceous-brown to sordid vinaceous brown in KOH, clamp connections present.

Gregarious on wet leaves in a springy area, under hardwoods, Colonial Point, Burt Lake, Cheboygan County, Michigan, July 31, 1947. Collected by Margaret Feigley (A.H.S. No. 26158—*type*).

Discussion. This is a very interesting species of the *C. ephemerus* series. The long narrow spores with the eccentric pore, the purplish color particularly in the stipes of young fruiting bodies, and habitat distinguish it from any other *Coprinus* known to me.

PANAEOLUS

PANAEOLUS ANOMALUS (Murrill) Saccardo & Trott. Syll. Fung. 23: 323. 1925. (FIGS. 26, 27—*type*.) Spores $11-13 (14) \times 6-$

$7 \times 7-9 \mu$, flattened, dark brown (darker than "warm sepia") when revived in KOH, narrowly subovate to elliptic in side view, broadly ovate in face view, some lopsided and some obscurely angled, apical hyaline pore present and distinct; basidia hyaline in KOH, four-spored, $18-22 \times 8-9 \mu$; paraphyses if present remaining collapsed; pleurocystidia abundant, $60-80 \times 10-20 \mu$, apices acute, the walls thickened particularly in the apices which are usually solid, deep yellowish brown in KOH, broadly fusoid to fusoid-ventricose, smooth; cheilocystidia similar to pleurocystidia but smaller (all hyaline thin-walled cells remained collapsed); gill trama sordid yellowish in KOH; pileus trama sordid yellowish in KOH, the cuticle of vesiculate cells and scattered fusoid-ventricose thin-walled hyaline pilocystidia scattered between the vesiculate cells.

Discussion. This species was described as *Campanularis anomalus* by Murrill (Mycologia 10: 32. 1918). Some authors place the species of *Panaeolus* with brown thick-walled pleurocystidia in a separate genus, *Copelandia*, but in my estimation the group is better regarded as a section of *Panaeolus*.

Panaeolus castaneifolius (Murrill) comb. nov. (FIG. 28-type.) Spores $12-16 \times 7-8.5 \mu$, somewhat almond-shaped, obscurely to distinctly verrucose, dark tawny to pale russet when revived in KOH, hyaline apical germ pore present but small and inconspicuous under ordinary magnifications; basidia $24-28 \times 10-12 \mu$, hyaline in KOH, four-spored; pleurocystidia present as dark cinnamon-brown basidia-like or narrower bodies embedded in the hymenium, $18-24 \times 6-10 \mu$; cheilocystidia abundant, fusoid-ventricose to subcylindric, $24-38 \times 7-10 \mu$, neck often flexuous and apices usually obtuse, thin-walled and hyaline; gill trama parallel to subparallel, dull cinnamon-brown when first revived in KOH; pileus trama with a cuticle of clavate, pear-shaped and vesiculate cells arranged into a somewhat irregular palisade, flesh proper floccose, interwoven and pallid to pale brownish when revived in KOH.

Discussion. Microscopically this species is almost identical with *Panaeolus foenesicii*, but the stipe, which is 4-6 mm. thick, the strong odor and unpleasant taste should distinguish it readily in the field. Murrill originally published it as *Psilocybe castaneifolia* (Mycologia 15: 17. 1923).

Panaeolus fraxinophilus sp. nov. (FIGS. 29-31-*type*.)

Pileus 8-15 mm. latus, conicus demum convexus, siccus et canescens demum udus et hygrophanus, fuscus vel niger, ad marginem Isabellinus; lamellae confertae, latae, cinereae demum nigro-maculatae; stipes 1-2 cm. longus, 1-2 mm. diam., cavus, fragilis, pruinosis, griseo-brunneus; sporae 9-11 \times 5-6 \times 6.5-7.5 μ . Ad. truncos *Fraxini*.

Pileus 8-15 mm. broad, conic to convex, surface appearing dry and hoary, typically becoming moist and hygrophanous, margin faintly translucent-striate and incurved at first, "fuscous black" to "fuscous" at first, nearly black on disc but soon "Benzo brown," "hair brown" to "drab" (dark gray), at or near margin "tawny olive" to "Isabella color" (sordid yellowish brown); flesh thin, dark, odor slightly farinaceous, taste mild, lamellae, close, moderately broad, adnate, dull drab becoming black-spotted, edges whitish; stipe 1-2 cm. long (up to 8 cm. around stumps), 1.5-2 mm. diam., hollow, fragile, equal, dark grayish brown with a tinge of red, densely pruinose over all and beaded with drops of moisture.

Spores 9-11 \times 5-6 \times 6.5-7.5 μ , smooth, slightly flattened, narrowly elliptic in side view, ovate in face view, dark reddish brown in KOH, black in mass, apical hyaline pore very small but distinct; basidia four-spored, a few two-spored, hyaline in KOH, 18-22 \times 9-10 μ , clavate; paraphyses not differentiated; pleurocystidia none; cheilocystidia very abundant, filamentous and apparently with mucilaginous walls, hyaline, many crooked, 28-42 \times 4-5 μ ; gill trama regular, pale dull brown in KOH; pileus trama with a dull brown region beneath the cuticle, remainder paler, cuticle of a layer of vesiculose hyaline cells one to two cells deep, numerous hyaline ventricose to subcylindric pilocystidia projecting and measuring 20-44 \times 5-6 μ , filamentous in age.

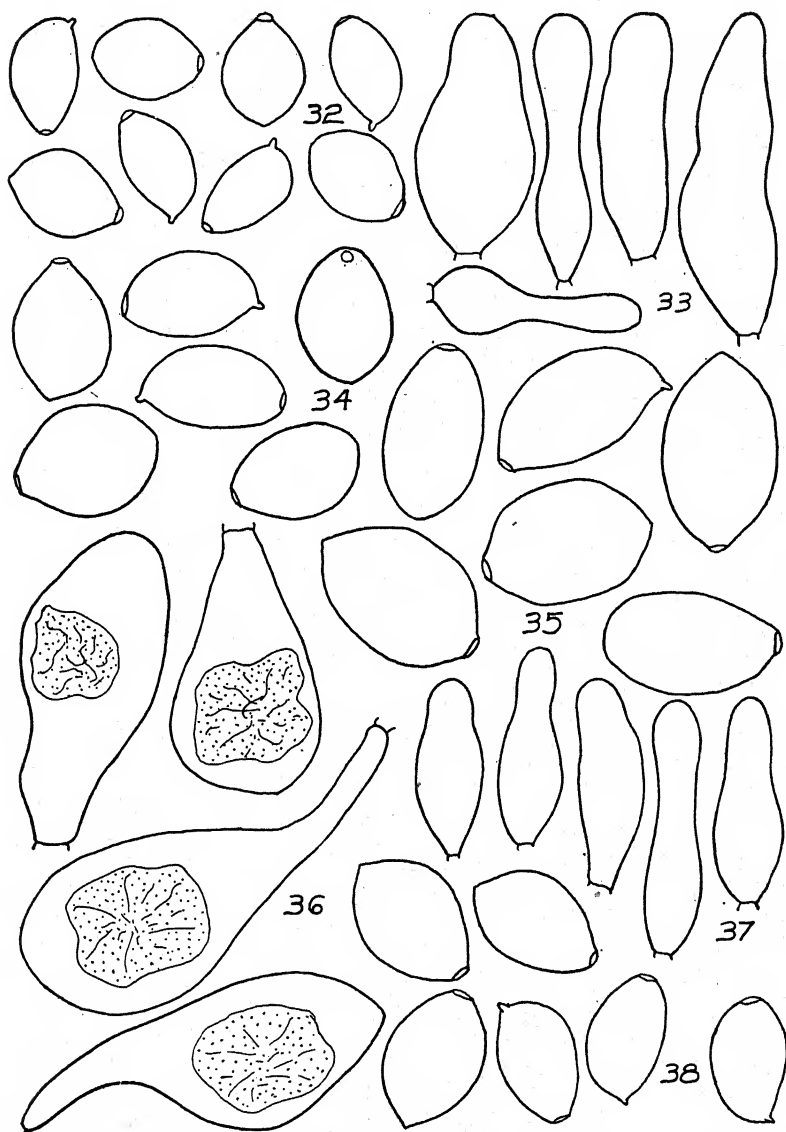
On naked trunk of a fallen ash tree, Warrensburg, New York, September, 1934, A. H. Smith 778-*type*.

Discussion. The combination of relatively small spores and habitat distinguish it from any *Panaeolus* known to me.

PANAEOLUS RETICULATUS Overholts, Ann. Mo. Bot. Gard. 3: 195. 1916. (FIG. 32-*type*.) Spores 9-11 (12.5) \times 5.6-6.2 \times 6.5-8 μ , slightly flattened, subelliptic to slightly inequilateral in side view, in face view ovate or a few very obscurely angular, dark bistre revived in KOH, apical hyaline pore small but distinct; basidia four-spored, hyaline when projecting but the collapsed hymenium and subhymenium bistre in KOH; pleurocystidia none; cheilocystidia fusoid-ventricose with obtuse apices, 18-26 \times 8-19 μ , gill trama bistre in KOH; pileus trama bistre, cuticle not

reviving, apparently of collapsed vesiculose cells. The tissue of the gills and pileus revived poorly.

Discussion. The spores separate it easily from *P. retirugis* and the gray to umber colors from *P. subbalteatus*.



FIGS. 32-38. Microscopic characters of dark-spored agarics.

PANAEOLUS RUFUS Overholts, Ann. Mo. Bot. Gard. 3: 196. 1916. (FIGS. 33, 34—*type*.) Spores $12-14 \times 6.5-8 \times 7-9.5 \mu$, slightly flattened, subovoid to subelliptic in side view, broadly ovate in face view, dark bister in KOH, hyaline pore apical to very slightly eccentric and inconspicuous; basidia hyaline in KOH (some collapsed basidia brownish), four-spored or a few two-spored and then sterigmata abnormal, $20-24 \times 10-12.5 \mu$; paraphyses apparently coprinoid but readily collapsing and basidia so numerous as to obscure them (hymenium in thick sections pale bister); pleurocystidia none; cheilocystidia abundant, hyaline in KOH, thin-walled, fusoid-ventricose to subvesiculose or subcylindric with a broadly rounded slightly enlarged apex, $26-42 \times (8) 10-14 \mu$, in age becoming elongated and with flexuous walls; gill trama parallel, the cells broad and long ($60-120 \times 15-20 \mu$), pale bister near and in subhymenium or finally sordid yellowish as revived in KOH; pileus trama sordid yellowish in KOH, cuticle of somewhat enlarged cells but not sharply distinct (as revived) from the remainder of the flesh.

Discussion. This species appears to me to be a synonym of *Panaeolus subbalteatus* as the latter is now understood. The only key character to separate them is the farinaceous odor and taste.

PANAEOLUS SEMIOVATUS (Fries) Lundell, Fung. Exsic. Upsal. No. 537. 1937. Spores $16-22 (23) \times 8.5-11 \mu$, smooth, apices truncate from a hyaline pore, ellipsoid in side view, slightly lemon-shaped to obscurely angled in face or back view, black in mass and dark blackish brown under microscope; basidia four-spored, $32-34 \times 12-14 \mu$; pleurocystidia rare to scattered, clavate to saccate or sometimes mucronate and with an irregular highly refractive content as revived in KOH (cystidia naematolomoid), $28-36 \times 10-18 \mu$; cheilocystidia similar to pleurocystidia or merely fusoid-ventricose and with homogeneous content, thin-walled, hyaline, $26-38 \times 7-12 \mu$, apices obtuse, walls sometimes flexuous; gill trama apparently regular (not reviving well); pileus trama with a cuticle of inflated hyaline cells several cells deep but very soon gelatinous and their outlines difficult to ascertain.

Discussion. Strictly speaking this fungus is not within the scope of this paper, but is included in order that its microscopic details may be more readily discussed in relation to those of other species. It is evident, from a consideration of the aspect of the fruiting bodies and the similarity of the microscopic characters, that this fungus and *P. solidipes* Peck are very closely related.

The similarity in the pleurocystidia of each emphasizes this point. *P. semiovatus* was placed in a separate genus, *Anellaria* (*A. separata* of Karsten), primarily because of the annulate stipe. Lange placed it in *Stropharia*. In view of the similarity in all characters except the annulus between *P. semiovatus* and *P. solidipes* it seems more sensible to me to retain both in the same genus and group them together in a distinct section. A veil is present in many species of *Panaeolus* but in most the remains adhere to the margin of the cap when the latter expands.

PANAEOLUS SOLIDIPES Peck, Ann. Rept. N. Y. State Mus. 23: 101. 1872. (Figs. 35–36–*type*.) Spores (12.5) $14-17 \times 6-8 \times 8-11 \mu$, flattened, narrowly subelliptic in side view, broadly ovate to obscurely angular-elliptic in face view, very dark bister to blackish in KOH, apical pore hyaline but very small and inconspicuous; basidia four-spored; hyaline in KOH, $20-26 \times 12-13 \mu$; paraphyses not differentiated; pleurocystidia scattered to abundant, $36-54 \times 10-20 \mu$, clavate from a narrow or a short, broad pedicel, hyaline in KOH and with an amorphous mass of a highly refractive substance in the enlarged portion, thin-walled and readily collapsing; cheilocystidia vesiculose, $18-24 \times 9-14 \mu$, also with a highly refractive content revived in KOH; gill trama regular, sordid yellowish as revived in KOH, pileus trama hyaline to sordid yellowish; cuticle of a single row (in sections) of hyaline inflated cells.

Discussion. See *P. semiovatus*.

PANAEOLUS VARIABILIS Overholts, Ann. Mo. Bot. Gard. 3: 197. 1916. (Figs. 37–38–*type*.) Spores $11-12 \times 6-6.6 \times 7.5-9 \mu$, flattened, subovate to slightly inequilateral in side view, ovate in face view, smooth, not obscurely angular, bister to dark bister as revived in KOH, hyaline apical pore distinct but not large; basidia hyaline in KOH, four-spored (a few with smoky brown sterigmata and some with brownish walls—the latter collapsed), $20-24 \times 10-12 \mu$; paraphyses not distinctly differentiated until late maturity and then readily collapsing (young basidia also somewhat saccate); pleurocystidia none; cheilocystidia abundant, hyaline and thin-walled, fusoid-ventricose to subcylindric, $26-35 \times 6-10 \mu$, elongated and crooked in age, apices obtuse to subcapitate; gill trama bister in KOH; pileus trama with a cuticle of inflated cells about one or two cells deep, these soon collapsing and difficult to demonstrate, flesh proper hyaline to sordid yellowish as revived in KOH.

Discussion. *P. variabilis* most closely resembles the fungus currently known as *P. papilionaceus*, but differs in smaller spores. It needs further study.

PANAEOLUS VENENOSUS Murrill, Mycologia 8: 186. 1916. (FIGS. 39, 40—*type*.) Spores $10-12.6$ (13) \times $6-6.5 \times 7-8.5 \mu$, dark bister revived in KOH, smooth, flattened, subovoid to elliptic in side view, not obscurely angular, apical hyaline pore small but distinct; basidia hyaline in KOH when isolated, brownish in thick sections, $18-24 \times 7.5-8.5 \mu$, clavate; paraphyses not distinctive; pleurocystidia present only near gill edges and similar to cheilocystidia; cheilocystidia fusoid-ventricose, $26-38 \times 6-10 \mu$, with a long, often crooked neck above a slightly ventricose basal portion, hyaline or bases brownish in KOH, thin-walled, apices capped with mucilage in some; gill trama bister in KOH, gradually becoming paler; pileus trama dark bister but gradually paler, cuticle formed by a palisade of clavate-pedicellate cells the bases of which are yellowish in KOH.

Discussion. In my estimation this species is not sufficiently distinct from *P. subbalteatus* to be recognized. The marginal belt in *P. subbalteatus* is not a constant character, in fact it is not visible on fresh entirely moist caps or on those completely faded. The spores in Murrill's type are slightly smaller than my measurements for *P. subbalteatus*, but the difference is too slight to be considered important until established from numerous spore deposits.

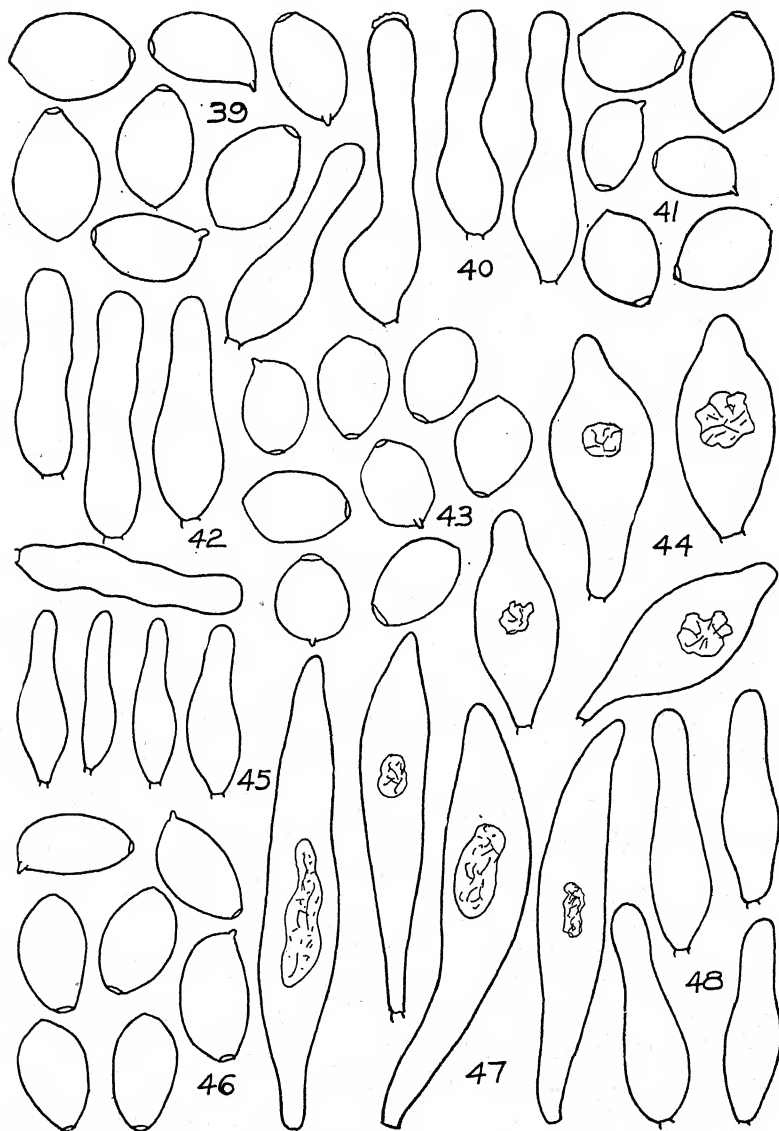
Panaeolus fontinalis sp. nov. (FIGS. 41, 42—*type*.)

Pileus 1-2 cm. latus, obtusus demum late conicus, impolitus vel pruinosis, griseo-olivaceus demum olivaceo-brunneus; lamellae pallide olivaceae demum cinereae et maculatae, confertae, latae, adnatae; stipes 5-10 cm. longus, 1-2 mm. crassus, fragilissimus, pallidus, deorsum argillaceus; sporae $7-9 \times 4-5 \times 5-6.5 \mu$.

Pileus 1-2.5 cm. broad, obtusely conic, expanding to broadly conic, surface unpolished to pruinose, "deep olive buff" over disc, paler toward margin, with a "buffy olive" marginal belt, finally "olive brown" or darker over all, fading eventually to "light grayish olive," with a silky appearance in age; flesh very thin and pallid, odor not distinctive; lamellae near "olive buff" young, soon drab, finally mottled with black, ascending adnate, close, broad, usually with 2 tiers of lamellulae, edges at first whitish and slightly floccose; stipe 5-10 cm. long, 1-2 mm. diam., equal, very fragile,

pallid and densely pruinose at apex, darker downward and "cinnamon buff" to "clay color" or tawny olive, pruinose over all at first and beaded with drops, naked in age.

Spores black in deposits, dark bistre in KOH, $7-9 \times 4-5 \times 5-6.5 \mu$, smooth flattened, broadly ovate in face view, nearly elliptic



FIGS. 39-48. Microscopic characters of dark-spored agarics.

in side view, pore apical and distinct; basidia four-spored, $18-24 \times 7-8.4 \mu$, hyaline in KOH; pleurocystidia absent to scattered (often abundant where insects have damaged hymenium), similar to cheilocystidia; cheilocystidia fusoid-ventricose to subcylindric, $26-34 \times 6-9 \mu$, apices obtuse, neck often flexuous; gill trama pale bister in KOH, the elements more or less interwoven; pileus trama darker than the gill trama, cuticle of a single layer of vesiculose cells, pilocystidia present and similar to cheilocystidia or filamentous and $15-30 \times 4-6 \mu$, hyaline.

Scattered on black muck among liverworts and a species of *Mnium* in a springy area in a cedar swamp, Burt Lake, Cheboygan Co., Mich., during July, 1947. July 4, 25437; July 5, 25493, 25494 and 25495. Collection 25437 is designated the **type**.

Discussion. The habitat of this *Panaeolus* is very unusual. A careful search was made for rabbit dung or that of other small animals, but none was found. The habitat, very small spores, and olive buff gills characterize the species.

NAEMATOLOMA

Naematoloma campestre sp. nov. (Figs. 43-45-type.)

Pileus (6) 8-15 (20) mm. latus, obtuse conicus demum campanulatus vel plano-convexus, glaber, lubricus, subcastaneus demum pallide ferrugineus; lamellae latae, adnatae, subdistantes, subochraceae; stipes brevis, 2-3 cm. longus, 1-1.5 mm. crassus, subfibrillosus, deorsum fuscescens; sporae $7.8-9.3$ (10) \times $4.6-5 \times 5-6.7 \mu$.

Pileus (6) 8-15 (20) mm. broad, obtusely conic, expanding to broadly conic or subcampanulate, finally convex to nearly plane, surface glabrous or with a faint fringe of marginal fibrils when young, lubricous when moist, color "Vernona brown" to "warm sepia" or "auburn" to "pecan brown" on disc, fading first to "pale pinkish buff" or "cinnamon buff" (pale ferruginous), but in age darkening to pale tan, margin striate when moist; flesh pliant, thin, odor very faintly fragrant, taste mild to slightly bitterish; lamellae very broad, horizontally adnate with a decurrent tooth, readily seceding, subdistant (15-18 reach stipe), one tier of lamellulae, dull brown becoming near "snuff brown" to very sordid cinnamon-brown, edges even and not whitish; stipe short, 2-3 cm. long, 1-1.5 mm. thick, equal, cartilaginous-pliant, about "light pinkish cinnamon" over all at first from thin remains of light pinkish cinnamon veil, soon darkening from base upward to concolor or darker than the cap, only somewhat glabrescent.

Spores 7.8–9.3 (10) \times 4.6–5 \times 5–6.7 μ , flattened slightly, angular-subspheric to angular-ovate or subelliptic with sides \pm parallel and with faintly angled shoulders in face view, subelliptic in side view, pore apical but small, pale bister as revived in KOH; basidia four-spored, hyaline in KOH, 18–23 \times 7–8 μ , subcylindric; paraphyses basidioid; pleurocystidia nearly embedded in hymenium and originating from gill trama or subhymenium, ventricose-mucronate to fusoid-ventricose and with a highly refractive amorphous body as seen revived in KOH (*Naematoloma* type), 26–34 \times 9–14 μ ; cheilocystidia hyaline, thin-walled, homogeneous, 18–26 \times 4–7 μ , subcylindric to subfusoid-ventricose with obtuse apices; gill trama of subparallel hyphae, pale to rather dark bister as revived in KOH; pileus trama dark bister to sordid cocoa-color in KOH, homogeneous (no differentiated hypoderm or pellicle).

Gregarious on sod in pastures where *Agaricus campestris* is frequently found, definitely not on dung. May 24, 1946, Smith 21426-type.

Discussion. The spore characters and habitat are more like *Psilocybe*, but the pleurocystidia are typical of *Naematoloma*.

Naematoloma humidicola (Murr.) comb. nov. (FIGS. 46–48-type.) Spores 9–11 \times 5.5–6.3 μ , ovoid in front or back view, ellipsoid to slightly inequilateral in side view, pale tawny in KOH, smooth, apical pore present but very indistinct; basidia hyaline to pale yellow in KOH, four-spored, subcylindric, 23–27 \times 5–6.5 μ ; pleurocystidia scattered, yellow in KOH, fusoid ventricose to subclavate-mucronate, (44) 50–58 (64) \times 8–10 (12) μ , most of them with a highly refractive content, some with what appears to be a wrinkled inner wall, projecting 25–30 μ beyond the basidia; cheilocystidia 24–28 (32) \times 5–8 μ , ventricose to subcylindric, hyaline, content homogeneous, thin-walled, abundant; gill trama subparallel, the cells equal in width throughout their length, pale yellow in KOH, subhymenium cellular but very thin and indistinct, pileus trama with a thin pellicle of narrow gelatinous hyphae 2.5–4 μ in diam.; beneath the pellicle is a region of enlarged cells (the hypoderm), the remainder of the flesh filamentous-floccose, all parts pale yellow in KOH, clamp connections present.

Discussion. Murrill described this species as *Naucoria humidicola* (North Am. Flora 10: 174. 1917). However, its natural affinities are unquestionably in the vicinity of *Naematoloma elongatipes*. It is distinguished among the slender species of *Naematoloma* by the conspicuous, elongated (for this genus) pleuro-

cystidia. In most species in this genus the cystidia do not project appreciably beyond the hymenium.

Naematoloma petasiforme (Murrill) comb. nov. (Figs. 49–51–*type*.) Spores $8-10 \times 5-6 \mu$, ellipsoid to subovoid, smooth, smoky purple-brown under the microscope when fresh (Murrill), sordid to pale tawny when revived in KOH, with an apical hyaline germ pore; basidia four-spored, subcylindric to clavate, hyaline in KOH, $20-24 \times 7-8 \mu$; pleurocystidia $20-30 \times 9-12 \mu$, mucronate, clavate or fusoid-ventricose and with a highly refractive body of amorphous material in enlarged part; cheilocystidia similar to pleurocystidia or fusoid-ventricose and $20-32 \times 8-11 \mu$ with a hyaline homogeneous content, the former rare, the latter very abundant and also occurring near gill edge as pleurocystidia; gill trama regular, tawny brown in KOH, hyphae somewhat interwoven and with encrusted pigment; pileus trama with a cuticle of radially arranged hyphae mostly $4-6 \mu$ in diam. and with clamp connections, the walls with encrusting pigment, some cells of the cuticle barrel-shaped and up to 15μ in diam. (but no true cuticle of vesiculate cells visible in type), flesh proper floccose and golden tawny in KOH.

Discussion. Murrill described this as *Psathyrella petasiformis* (Mycologia 14: 276. 1922). It differs from other members of the *Naematoloma dispersum* series by its lack of a thin pellicle. There are other less striking differences such as the slightly larger spores and possibly a darker spore deposit. However, these cannot be given much emphasis until comparative studies of fresh material can be made and spores from deposits compared.

PSILOCYBE

Psilocybe bulbosa (Pk.) comb. nov. (Figs. 52, 53–*type*.) Spores $6-7.5 (8) \times 3.8-4.2 \times 4-4.8 \mu$, angular-ovate in face view, pointed at base, slightly inequilateral to subovoid in side view, flattened, dull yellowish brown when revived in KOH, with an obscure apical hyaline pore; basidia $20-24 \times 6-7 \mu$, four-spored, hyaline in KOH; pleurocystidia embedded and difficult to locate, $18-24 \times 6-7 \mu$, obtusely fusoid-ventricose, hyaline in KOH, content homogeneous; cheilocystidia abundant, $20-28 \times 4-8 \mu$, similar to pleurocystidia or more elongated, when revived in KOH often with a drop of a mucilaginous substance adhering to apex; gill trama parallel, hyaline to pale brownish in KOH; pileus trama with a thin, hyaline gelatinous pellicle of hyphae $1.5-3 \mu$ in diam.,

and bearing clamp connections, flesh proper of compactly interwoven hyphae, pallid tawny brownish in KOH.

Discussion. The distant gills, angular to ovate spores in face view, and thin gelatinous pellicle appear distinctive. I suspect the



FIGS. 49-65. Microscopic characters of dark-spored agarics.

pale color of the cap as described by Peck was caused by fading. The species was described as *Deconica bulbosa* by Peck (Ann. Rep. N. Y. State Mus. 46: 107. 1893).

PSILOCYBE CAESPITOSA Murrill, Mycologia 15: 5. 1923. (Figs. 54, 55—*type*.) Spores $6.2-7.5 \times 4-5.2 \mu$, smooth, subelliptic in side view, obscurely angular to ovate in face view, terete to very slightly compressed, pale ochraceous tawny when revived in KOH, truncate from a small apical germ pore; basidia four-spored, $16-20 \times 6-7 \mu$, hyaline in KOH; pleurocystidia none; cheilocystidia abundant, $22-28 \times 3-6 \mu$, narrowly fusoid, hyaline; gill trama parallel or nearly so, regular, the hyphae $4-8 \mu$ in diam., sordid yellowish to hyaline in KOH; pileus trama homogeneous beneath a thin hyaline gelatinous pellicle of hyphae $2-4 \mu$ in diam., flesh proper floccose and sordid yellowish in KOH, clamp connections present.

Discussion. This species is very closely related to *P. subviscida* Peck, but differs sharply in the spacing of the gills. Those of Peck's species are subdistant instead of crowded. *P. caespitosa* apparently has a more shaggy-fibrillose stipe and slightly smaller spores in addition, but the latter difference is very slight.

PSILOCYBE CASTANELLA Peck, Bull. N. Y. State Mus. 1: 7. 1888. (Figs. 56, 57—*type*.) Spores $6-7 \times 3.5-4 \mu$, subellipsoid to ovoid, not flattened, with a small hyaline apical pore, dull yellowish brown revived in KOH; basidia four-spored, $18-22 \times 6-7 \mu$, hyaline in KOH; pleurocystidia none; cheilocystidia $18-24 (28) \times 3-5 \mu$, hyaline in KOH, subventricose to nearly cylindric, apices obtuse to subacute, apex often with an adhering drop or cap of a hyaline mucilaginous substance; gill trama parallel, yellowish brown in KOH or more tawny next to the subhymenium; pileus trama homogeneous beneath a thin, hyaline, gelatinous pellicle, remainder yellowish brown or toward the subhymenium paler.

Discussion. *Psilocybe californica* Earle (Bull. N. Y. Bot. Gard. 3: 301. 1904) appears, beyond the possibility of a doubt, to be this species. Its spores are also illustrated (FIG. 59). The habitat on sod appears to be distinctive as is also the habit of growing in rather dense masses. The latter feature is more characteristic of specimens from California. The cheilocystidia (FIG. 58) are also similar.

PANAEOLUS DIGRESSUS Peck, Bull. Torrey Club 22: 205. 1895. Spores sordid yellowish brown in KOH, $12-14 \times 6.8-7.8 \times 8-9 \mu$,

slightly compressed, elliptic in side view, obscurely angled and broadly elliptic in outline in face view, hyaline apical pore small but distinct; basidia $30-35 \times 10-12.5 \mu$, clavate, hyaline in KOH, four-spored; paraphyses not distinct from young basidia; pleurocystidia none, sections of hymenium pale sordid yellowish revived in KOH; cheilocystidia forming a sterile band on gill edge, hyaline, thin-walled, often crooked to contorted, typically fusoid-ventricose to subcylindric, $18-32 \times (4) 5-8 \mu$, apices obtuse and some capped with mucilage; gill trama pale sordid yellowish in KOH, subparallel next to subhymenium, central strand interwoven; pileus trama pale sordid yellowish in KOH except for a more tawny colored region just below the gelatinous cuticle, the hyphae of the cuticle hyaline to yellowish and $2-3 \mu$ in diam., those of flesh proper $6-8 (10) \mu$ in diam.

Discussion. A synonym of *Psilocybe coprophila*.

Psilocybe fuliginosa (Murrill) comb. nov. (FIGS. 60, 61-type.) Spores $6-7 \times 4.5-5.5 \mu$, smooth, typically triangular in optical section but varying to ovoid or ellipsoid (as seen in face view), ellipsoid in side view, sordid yellowish brown when revived in KOH, with an obscure apical hyaline germ pore; basidia four-spored, $18-20 \times 5-6 \mu$, hyaline in KOH; pleurocystidia scattered to rare, similar to cheilocystidia; cheilocystidia $16-28 \times 4-7 \mu$, hyaline, narrowly ventricose, the neck flexuous and the apex acute to subacute or in the form of a small knob, many with a highly viscous substance adhering near or on the apex; gill trama parallel, sordid yellowish brown in KOH; pileus trama homogeneous; no pellicle seen, upper region darker yellowish brown in color than that near subhymenium.

Discussion. Dr. Burke has sent me material from Alabama which appears to belong here though the spores vary slightly, being somewhat longer. They range from 6.5 to 8μ . For *P. fuliginosa* the habitat on bare earth, the dry pileus, shape of spores in face view, and fuliginous cast of the carpophore are apparently distinctive. Murrill described it as *Athylospora fuliginosa* (Mycologia 10: 25. 1918).

PSILOCYBE GRAVEOLENS Peck, Bull. N. Y. State Mus. 167: 47. 1913. (FIG. 62-type.) Spores $8-10 \times 5-6 \mu$, ellipsoid in face view, in side view subelliptic to slightly inequilateral, smooth, pale sordid ochraceous tawny under the microscope in KOH, apical pore small but distinct; basidia hyaline in KOH, $22-26 \times 6-7 \mu$, four-spored; pleurocystidia rare to scattered, fusoid, $28-32 \times 7-$

9 μ , the walls slightly brownish in some, hyaline in others when revived in KOH, buried in the hymenium; cheilocystidia hyaline, 12–22 \times 7–9 μ , necks 4–6 μ broad and contorted to merely flexuous; gill trama parallel, more or less ochraceous tawny when revived in KOH; pileus trama homogeneous, no pellicle differentiated, floccose, interwoven and pale to dark ochraceous tawny in KOH.

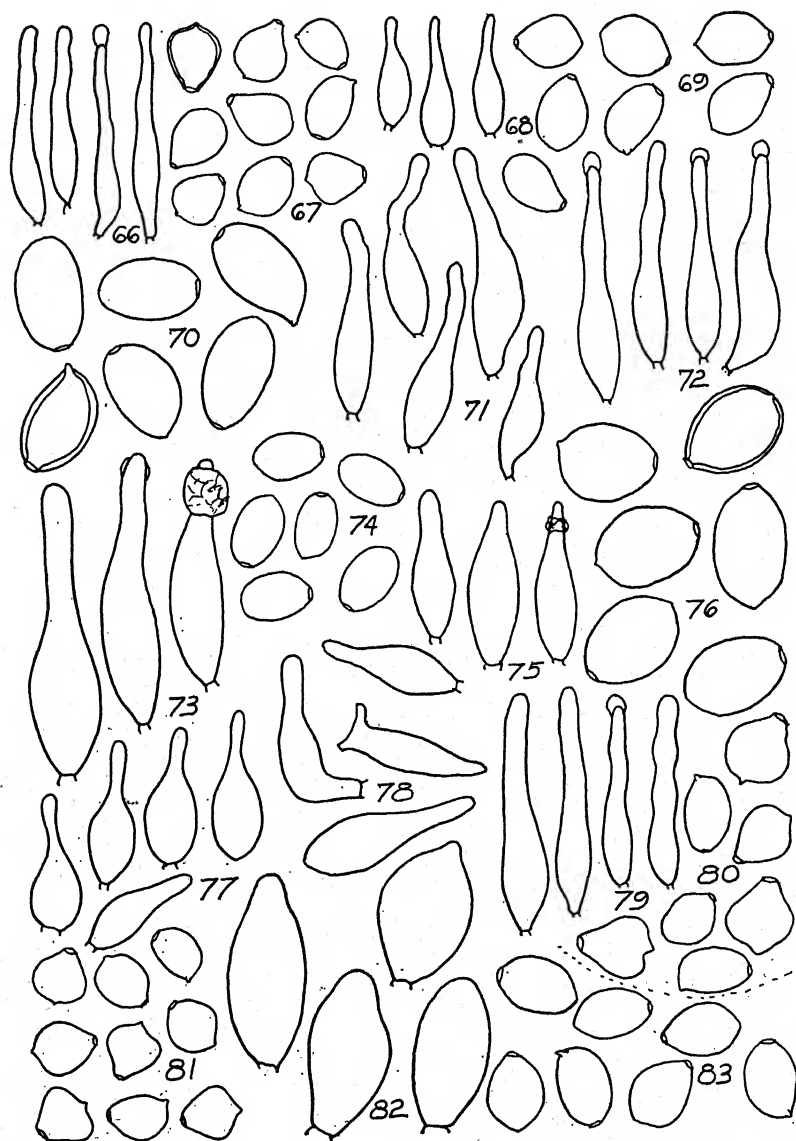
Discussion. The strong odor and clustered manner of growth on soil should be distinctive. The spores are almost identical with those of *P. silvatica*, and I suspect the species belongs in that group even though no bluish or greenish color is reported for it.

Psilocybe lateritia (Murrill) comb. nov. (Figs. 63, 64–type.) Spores ovoid to subellipsoid, terete or nearly so, 7.5–9.3 \times 5–6.2 μ , smooth, not angular, dull yellowish brown in KOH, apical pore present but very obscure; basidia 18–20 \times 6–7 μ , cylindric, hyaline or in thick sections yellowish, four-spored; pleurocystidia scattered, similar to cheilocystidia; cheilocystidia abundant, ventricose with acute apices but elongating to aciculate, 28–46 \times 6–9 μ (narrowest in the longest ones), neck or apex usually with an adhering mass of a highly viscous substance slowly soluble in KOH, content homogeneous and hyaline; gill trama of parallel hyphae, sordid pale yellowish in KOH; pileus trama with a thin cuticle of hyaline to pale yellowish gelatinous hyphae bearing clamp connections, beneath this a narrow region of cinnamon-brown hyphae (in KOH) but not otherwise distinct from the remainder of the flesh which is paler brownish to yellowish.

Discussion. This species was described as *Psathyra lateritia*, by Murrill (Mycologia 10: 33. 1918). It belongs in the group with *P. atrorufa*, but since this group is in need of a critical study, no attempt at further comparisons will be made here. It is not identical with any of my collections in that group.

PSILOCYBE LATISPORA Murrill, Mycologia 15: 10. 1923. (Figs. 65, 66–type.) Spores 6.2–7.8 \times 4.6–5.2 \times 6–6.2 μ , flattened, subovate to subcircular in outline in face view, often slightly angular, in side view slightly inequilateral to subelliptic, dark dull yellowish brown in KOH, apex truncate from an apical hyaline pore; basidia 18–20 \times 7–8 μ , hyaline in KOH, four-spored; pleurocystidia none; cheilocystidia 22–26 \times 4–7 μ , aciculate to narrowly ventricose, apex at times with a hyaline globule; gill trama regular, hyphae parallel to somewhat interwoven, with dull yellowish brown

walls in KOH; pileus trama homogeneous, surface hyphae slightly gelatinous in KOH but no true pellicle present, floccose, compactly interwoven and with dull yellowish brown walls when revived in KOH.



FIGS. 66-83. Microscopic characters of dark-spored agarics.

Discussion. This species is apparently closely related to *P. phyllogena* Peck but differs in its larger spores.

***Psilocybe mammillata* (Murr.) comb. nov.** (Figs. 67, 68-type.) Spores $5-6$ (7) \times $4.5-5\ \mu$, smooth, triangular in face view, some ovate-angular, in side view subelliptic to ovate, dull ochraceous tawny revived in KOH, with a small apical pore; basidia hyaline in KOH, $13-15 \times 5-6\ \mu$, four-spored; pleurocystidia similar to cheilocystidia; cheilocystidia small and inconspicuous, $12-18 \times 4-8\ \mu$, fusoid-ventricose or the apices subcapitate, hyaline; gill trama parallel, pale yellowish bister from a pigment which encrusts the hyphae, darker near subhymenium; pileus trama homogeneous, the hyphae near the surface a darker yellowish brown (bister) than those toward the subhymenium, clamp connections present.

Discussion. Murrill described the fungus as *Psathyra mammillata*, (*Mycologia* 10: 33. 1918). The spores and cheilocystidia are distinctive. *Psathyra cinchonensis* Murrill (*Mycologia* 10: 33. 1918) is identical microscopically with *P. mammillata* and in the dried specimens the gill spacing did not appear distinctive. *Psathyra cinchonensis* is therefore considered a synonym of *P. mammillata*.

***Psilocybe modesta* (Peck) comb. nov.** (Figs. 69; 72-type.) Spores $5.5-6.3 \times 3.6-4.2 \times 4-4.3\ \mu$, subtriangular to broadly ovate in face view, elliptic in side view, pale bister to sordid tawny revived in KOH, apex truncate because of a small hyaline germ pore; basidia hyaline in KOH, four-spored, $14-18 \times 4.5-5.5\ \mu$; pleurocystidia only near gill edge and similar to cheilocystidia; cheilocystidia abundant, hyaline, $22-26 \times 3-5\ \mu$, narrowly ventricose, often with drops of a mucilaginous substance adhering to apex; gill trama regular to somewhat interwoven and colored like the pileus trama or paler; pileus trama with a thin poorly formed pellicle of subgelatinous hyphae (in KOH), flesh proper floccose and dark, bright cinnamon in KOH (pale in very thin sections), pigment loosely encrusted on cell walls.

Discussion. In its small spores it resembles *P. mammillata* but the veil is pronounced and the cheilocystidia much longer.

PSILOCYBE NIGRELLA Peck, Bull. N. Y. State Mus. 139: 28. 1910. (Figs. 70, 71-type.) Spores $9-11$ (12) \times $6-7.5$ (8) μ , dark sordid yellowish brown revived in KOH, smooth, terete, broadly ovoid to ellipsoid, apical pore small and obscure; basidia

four-spored, $22-26 \times 7-8.5 \mu$, hyaline in KOH; pleurocystidia none seen (some collapsed basidia brown); cheilocystidia abundant and forming a sterile band on the gill edge, hyaline, narrowly ventricose to almost aciculate, $20-26 \times 4-7 \mu$, apices often somewhat flexuous; gill trama of long broad parallel cells with parallel walls, pale rusty brown in KOH, subhymenium cellular and dark rusty brown, the pigment encrusted on the cell walls; pileus trama with a gelatinous pellicle of narrow ($2-4 \mu$) hyaline appressed hyphae, beneath this a zone of dark yellowish brown hyphae with the pigment encrusted on the walls, paler toward the subhymenium, the hyphae interwoven and all with broad cells ($8-15 \mu$ in diam.).

Discussion. The short stipe is the only character I can find to distinguish it from *P. atrobrunnea*, and length of stipe is a very variable character in bog-inhabiting species.

Psilocybe pallidispora (Murrill) comb. nov. (Figs. 73-75-type.) Spores $5.3-6.2 \times 3.5-4.2 \mu$, terete, ellipsoid to ovoid, with a very small apical pore, dull yellowish brown in KOH; basidia $16-20 \times 5-6 \mu$, four-spored, hyaline in KOH, pleurocystidia scattered, $34-44 \times 7-10 \mu$, fusoid-ventricose with elongated necks and acute apices, the apex or neck frequently with a drop or coating of a highly viscous substance adhering to it, hyaline in KOH, thin-walled, content homogeneous; cheilocystidia $22-28 \times 5-7 \mu$, fusoid-ventricose, in some subcapitate, hyaline in KOH, often with mucilaginous cap; gill trama regular, dark brown (near cinamon-brown) when revived in KOH; pileus trama homogeneous, no pellicle seen, surface region a darker brown than that toward the subhymenium, pigment encrusted on the walls.

Discussion. Distinct by virtue of the small ellipsoid spores and moderately large pleurocystidia. The pileus is typically dry.

PSILOCYBE PANAEOLIFORMIS Murrill, Mycologia 15: 12. 1923. (Figs. 76-78.) Spores $9-11.5 \times 6-7 \mu$, smooth, ellipsoid to sub-ovoid, a few obscurely angular, not compressed, sordid yellowish brown to pale bistre revived in KOH, apex truncate from a hyaline apical pore; basidia four-spored, short and fat, $14-16 \times 8-9 \mu$, hyaline in KOH; pleurocystidia none seen; cheilocystidia very abundant and forming a sterile band on gill edge, $12-16 \times 4-8 \mu$, ventricose and with a slender flexuous neck, apices subacute, hyaline in KOH; gill trama parallel to subparallel, pallid, brownish in KOH; pileus trama with a pellicle of narrow ($1.5-3 \mu$) filamentous hyaline hyphae subgelatinous in KOH, a few pilocystidia similar to cheilocystidia projecting from it, beneath this a hypo-

derm of enlarged cells cinnamon-brown in KOH, remainder of flesh also of enlarged hyphal cells but paler, clamp connections present on pellicle.

Discussion. This species appears to be a true *Psilocybe* and closely related to *P. coprophila*. It differs from the latter in terete somewhat narrower spores and short fat cheilocystidia. An imperfect was fruiting over the type and details of cap and gills could not be seen as clearly as desired. However, the data obtained were checked with the Alabama collection which is unquestionably the same.

PSILOCYBE PHYLLOGENA Peck, Bull. N. Y. State Mus. 157: 99. 1912. (FIGS. 79, 80—*type*.) Spores $5-6.5$ (7) \times $4-4.6 \times 5-6$ μ , more or less triangular in face view, often top-shaped or merely ventricose near base, subelliptic in side view, apical hyaline germ pore present causing apex to appear slightly truncate, when revived in KOH sordid tawny brown in color; basidia four-spored, hyaline in KOH, $18-22 \times 5-7$ μ ; pleurocystidia none or present only near the gill edge and similar to cheilocystidia; cheilocystidia narrowly fusoid, apices often with a mucilaginous cap in KOH, content hyaline and homogeneous, $22-26$ (34) \times $4-6$ (7) μ ; gill trama regular, parallel or nearly so, pallid brownish in KOH to nearly hyaline; pileus trama homogeneous, no true pellicle present but surface hyphae only slightly gelatinous, flesh proper pallid sordid brownish in KOH, finally becoming nearly hyaline, interwoven and floccose.

Discussion. The lack of a veil in addition to the small compressed spores appears to be distinctive, though the absence of a veil needs to be confirmed from an examination of numerous collections of young carpophores.

PSILOCYBE PLUTONIA (Berk. & Curt.) Saccardo, Syll. Fung. 5: 1056. 1887. (FIG. 81—*type*.) Spores $5-6.2 \times 3.8-4.2 \times 5-6$ μ , subtriangular in face view to angular-ovate or ventricose basally, often slightly indented around the apiculus, elliptic in side view, distinctly compressed, dull yellowish brown in KOH, with a small apical hyaline pore; basidia pale sordid yellowish to hyaline in KOH, $10-12 \times 5-6$ μ , four-spored; pleurocystidia and cheilocystidia not seen (no good sections of gill edges obtained); gill trama parallel, dark sordid yellowish brown in KOH; pileus trama homogeneous, dark yellowish brown (near bister) in KOH, no pellicle observed.

Discussion. This species appears to be in the *P. phyllogena* series and is distinct on spore characters alone.

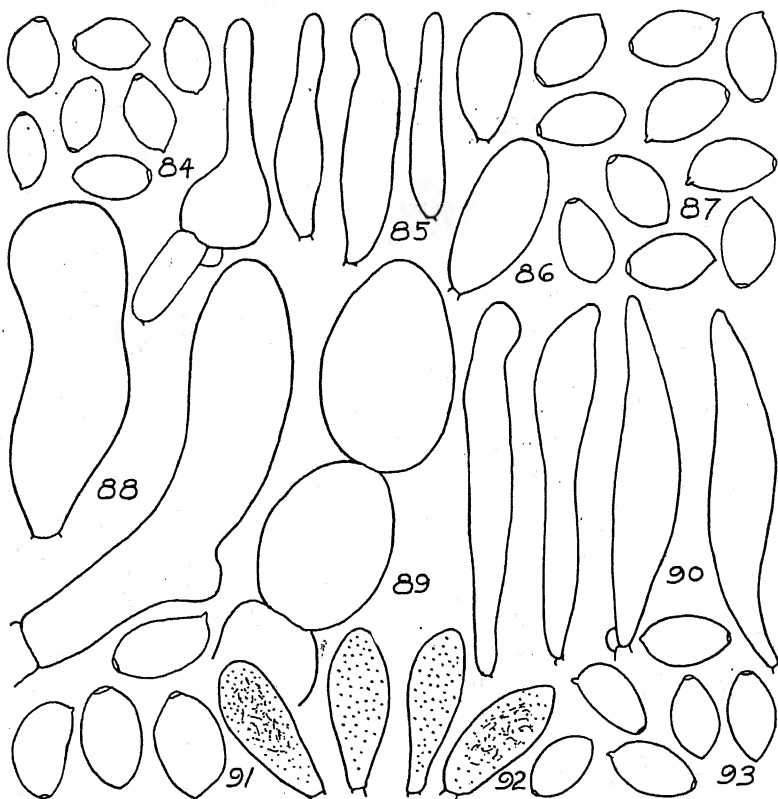
AGARICUS POLYTRICHOPHILUS Peck, Ann. Rep. N. Y. State Mus. 30: 42. 1878. (FIG. 83—*type*.) Spores $6-7.5 \times 4-5 \mu$, terete, ellipsoid to ovoid, smooth, not angular but an occasional spore ventricose at base, dull yellowish brown in KOH, apical pore present but small; basidia four-spored, $16-20 \times 6-7.5 \mu$, hyaline in KOH; pleurocystidia very rare and similar to cheilocystidia; cheilocystidia $16-23 \times 5-7 \mu$, narrowly fusoid-ventricose with obtuse apices, hyaline and with homogeneous contents when revived in KOH; gill trama parallel, yellowish brown; pileus trama with a thin gelatinous pellicle of hyaline hyphae, flesh proper compactly interwoven and yellowish brown in KOH.

Discussion. I am not sure at present as to the status of this species. Our common *Psilocybe* on *Polytrichum* has no odor and so I am not inclined to consider Peck's type identical with it.

Psilocybe pyrispora (Murrill) comb. nov. (FIGS. 82, 84—*type*.) Spores $6.5-7.5 \times 4-4.5 \mu$, smooth but some obscurely angular, terete, somewhat pear-shaped to ellipsoid or ovoid (angularity best seen in face view, dull yellowish brown when revived in KOH (pale smoky purplish brown in mounts of fresh material—Murrill), apical pore present but small; basidia four-spored, hyaline in KOH, $12-14 \times 6-7 \mu$; pleurocystidia scattered to fairly abundant, $18-26 (32) \times 10-15 \mu$, sessile or with a short pedicel, broadly ovate to elliptic in optical section, hyaline and highly refractive in KOH but content homogeneous, the walls only slightly thickened, smooth; cheilocystidia $18-26 \times 4-7 \mu$, narrowly ventricose to subcylindric, the necks straight or flexuous and apices often capped with mucilage, hyaline in KOH; gill trama not reviving well but apparently subgelatinous in KOH; pileus trama with a broad band of indistinct hyaline hyphae forming the upper region, lower region yellowish brown (upper portion apparently subgelatinous in KOH but details were not distinct).

Discussion. A very curious species by virtue of the pleurocystidia. It is the wall which refracts the light in the characteristic manner, and since the content of the cystidium is homogeneous and hyaline, the species cannot be properly regarded as belonging in *Naematoloma*. Murrill described it as *Deconica pyrispora* (Mycologia 14: 261. 1922).

PSILOCYBE SUBVISCIDA (Peck) Kauffman, Agar. Mich. p. 275. 1918. (*Deconica subviscida* Peck, Ann. Rep. N. Y. State Mus. 41: 70. 1888). (Figs. 85-87-type.) Spores $7-8 \times 4-4.5$ (5) μ , smooth, ellipsoid to somewhat ovoid, not appreciably angular, terete, yellowish brown in KOH, with a small apical germ pore; basidia $18-20 \times 6-7$ μ , four-spored, hyaline in KOH; pleurocystidia clustered between the gills, rare elsewhere, $34-50 \times 7-9$



FIGS. 84-93. Microscopic characters of dark-spored agarics.

(10) μ , ventricose, elongating to subcylindric with flexuous necks and obtuse apices, hyaline, thin-walled; cheilocystidia variable, ventricose to ellipsoid at first but soon developing a flexuous projection up to $30-40$ μ long and $6-7$ μ thick or fusoid-ventricose from the first, the former $20-26 \times 10-12$ μ at first, $36-50 \times 7-10$ μ in age, the others usually smaller and $20-32 \times 5-8$ μ ; gill trama regular or nearly so, color not distinctive; pileus trama with a

thin gelatinous pellicle of narrow hyaline hyphae, remainder compactly interwoven and floccose, not distinctively colored in KOH.

Discussion. The occurrence of pleurocystidia between the gills should not be given much emphasis. In many species such structures develop along the line where a new gill is forming and hence they could also be interpreted as cheilocystidia. I suspect that such is the situation here.

Psilocybe tomentosa (Murrill) comb. nov. (FIGS. 88-91-type.) Spores $8-10 \times 5-6 \mu$, smooth, slightly compressed to terete, angular to elliptic in face view, slightly bean-shaped to subelliptic in side view, some ventricose basally and with a median constriction (apparently abnormal), dull tawny revied in KOH, apical pore hyaline and very inconspicuous; basidia mostly four-spored, hyaline in KOH; $24-28 \times 5.5-7 \mu$; pleurocystidia rare and embedded in the hymenium, similar to cheilocystidia or shorter; cheilocystidia $34-48 \times 5-8 \mu$, subcylindric to subfusoid, the apices obtuse, broadest at or above the middle and neck very short and indistinct, a few with the apex embedded in a mucilaginous substance; gill trama parallel to somewhat interwoven, pale yellowish in KOH; cuticle of pileus consisting of a turf of upright pilocystidia and chains of \pm globose cells with a pale tawny encrusting pigment on the walls, the pilocystidia more or less clavate, $20-52 \times 8-12 \mu$ and irregularly arranged so as to project to varying distances above each other, hyaline or with encrusted pigment, the chains of vesiculose cells not highly colored in KOH (as they are in genus *Cystoderma*), masses of vesiculose cells and pilocystidia aggregated into fascicles to form scales on the cap; flesh of cap of interwoven hyphae which are pale yellowish in KOH, clamp connections present.

Discussion. Although the microscopic characters of this species depart radically from those of most *psilocybes* in respect to the cuticle of the pileus, the differences do not seem to me sufficient to justify erecting a new genus.

PSILOCYBE VIALIS Murrill, *Mycologia* 15: 11. 1923. (FIGS. 92, 93-type.) Spores pale yellowish in KOH, $7-8.4 \times 4-4.6 \mu$, terete, ellipsoid to somewhat ovoid, some obscurely angular in face view, apex truncate from a small hyaline germ pore; basidia $17-20 \times 5-6.5 \mu$, four-spored, hyaline in KOH; pleurocystidia embedded in hymenium or subhymenium, $20-26 \times 8-10 \mu$, clavate to obovoid above a thick pedicel, brownish in KOH and with a granular content or some with a highly refractive amorphous sub-

stance; cheilocystidia abundant, $20-26 \times 4-6 \mu$, acuminate to narrowly fusoid, hyaline, apex often with an adhering globule of a hyaline viscous substance; gill trama regular, the hyphae somewhat interwoven, pale ochraceous tawny in KOH; pileus trama lacking a distinct pellicle, surface region nearly hyaline, more or less ochraceous tawny toward the gills.

Discussion. The pleurocystidia are not those of *Naematoloma*, and I doubt if the species is any more closely related to members of that genus than are the other *psilocybes*.

EXPLANATION OF FIGURES

The illustrations of spores, cystidia, and veil elements were drawn with the aid of a camera lucida. As reproduced the spores are approximately $1650 \times$ natural size; the cystidia and veil elements are approximately $700 \times$ natural size unless otherwise stated.

FIGS. 1-9. *Coprinus angulatus*: 1, pilocystidia; 2, spores in side and face view. *Coprinus Brassicae*: 3, spores in side and face view. *Coprinus calyptratus*: 4, spores in both views. *Coprinus asterophorus*: 6, spores in both views. *Coprinus cinchonensis*: 5, cells from veil showing spines; 7, spores in both views. *Coprinus ebulbosus*: 8, spores—some showing ventral hump (or pore). *Coprinus hexagonosporus*: 9, spores in both views.

FIGS. 10-22. The spores are shown in both side and face views. *Coprinus insignis*: 10, spores. *Coprinus jalopenis*: 11, spores. *Coprinus Jonesii*: 12, spores. *Coprinus pseudoradiatus*: 13, spores. *Coprinus laniger*: 14, spores. *Coprinus mexicanus*: 15, spores. *Coprinus pulchrifolius*: 16, spores. *Coprinus quadrifidus*: 17, spores. *Coprinus rotundisporus*: 18, pleurocystidia; 19, spores. *Coprinus Seymourii*: 20, spores. *Coprinus semilanus*: 21, spores. *Coprinus silvaticus*: 22, spores.

FIGS. 23-31. The spores are shown in both side and face views. *Coprinus Spraguei*: 23, six spores. *Coprinus variegatus*: 24, ten spores, two of which show ventral hump or pore. *Coprinus subpurpureus*: 25, spores. *Panaeolus anomalus*: 26, spores; 27, pleurocystidia. *Panaeolus castaneifolius*: 28, spores. *Panaeolus fraxinophilus*: 29, spores; 30, pilocystidia; 31, cheilocystidia.

FIGS. 32-38. The spores are shown in both views. *Panaeolus reticulatus*: 32, spores. *Panaeolus rufus*: 33, cheilocystidia; 34, spores. *Panaeolus solidipes*: 35, spores; 36, pleurocystidia. *Panaeolus variabilis*: 37, cheilocystidia; 38, spores.

FIGS. 39-48. *Panaeolus venenosus*: 39, spores; 40, cheilocystidia. *Panaeolus fontinalis*: 41, spores; 42, cheilocystidia. *Naematoloma campestre*: 43, spores; 44, pleurocystidia; 45, cheilocystidia. *Naematoloma humidicola*: 46, spores; 47, pleurocystidia; 48, cheilocystidia.

FIGS. 49-65. *Naematoloma petasiforme*: 49, pleurocystidia; 50, spores; 51, cheilocystidia. *Psilocybe bulbosa*: 52, spores; 53, cheilocystidia. *Psilocybe caespitosa*: 54, spores; 55, cheilocystidia. *Psilocybe castanella*: 56, cheilocystidia; 57, spores. *Psilocybe californica*: 58, cheilocystidia; 59, spores. *Psilocybe fuliginosa*: 60, spores; 61, cheilocystidia. *Psilocybe*

graveolens: 62, spores. *Psilocybe lateritia*: 63, pleuro- and cheilocystidia; 64, spores. *Psilocybe latispora*: 65, spores.

FIGS. 66-83. *Psilocybe latispora*: 66, cheilocystidia. *Psilocybe mammilata*: 67, spores; 68, cheilocystidia. *Psilocybe modesta*: 69, spores; 72, cheilocystidia. *Psilocybe nigrella*: 70, spores; 71, cheilocystidia. *Psilocybe pallidispora*: 73, pleurocystidia; 74, spores; 75, cheilocystidia. *Psilocybe panaeoliformis*: 76, spores; 77, cheilocystidia; 78, pilocystidia. *Psilocybe phyllogena*: 79, cheilocystidia; 80, seven spores. *Psilocybe plutonia*: 81, spores. *Psilocybe pyrispora*: 82, pleurocystidia. *Agaricus polytrichophilus*: 83, spores.

FIGS. 84-93. *Psilocybe pyrispora*: 84, spores. *Psilocybe subviscida*: 85, cheilocystidia; 86, cheilocystidia; 87, spores. *Psilocybe tomentosa*: 88, pilocystidia; 89, veil elements; 90, cheilocystidia; 91, spores. *Psilocybe vialis*: 92, pleurocystidia; 93, spores.

AN UNDESCRIBED SPECIES OF HELMINTHOSPORIUM ON SUDAN GRASS AND SORGHUM

C. L. LEFEBVRE AND HELEN S. SHERWIN

(WITH 2 FIGURES)

In 1939, G. W. Burton collected and sent us (7) leaves of a common Sudan grass \times Leoti sorghum hybrid (now Tift Sudan grass), bearing a leaf spot that was defoliating some of his breeding material. Lesions on these leaves varied considerably, the smaller ones being 0.5 to 1.0 mm. in each direction, whereas the longer ones measured up to 15 mm. in length and 1 to 6 mm. in width. The lesions were round to elliptical, the long axis parallel to the leaf veins, and oftentimes the spots seemed to be limited in their spread by the veins. Many of the lesions had coalesced so that comparatively large portions of the leaves were affected (FIG. 1, A). The most characteristic features of the lesions, however, were the alternate light tan and darker, more narrow bands of tissue, producing a zonate appearance which suggests the common name "target spot" (FIG. 1, A). Lesions were usually surrounded by a narrow tannish-brown border setting off the affected area from the healthy tissue. The symptoms on Tift Sudan grass are strikingly similar to the lesions on corn and teosinte caused by the fungus originally described as *Ophiobolus heterostrophus* Drech. (5), but later transferred to the genus *Cochliobolus* (6).

Although the fungus causing the target spot of Tift Sudan grass has never been collected on common Sudan grass, the latter was found to be susceptible when inoculated. On common Sudan, however, the lesions appear less zonate (FIG. 1, B). The spots are often uniformly dark, or when older they may have a straw-color center surrounded by a reddish-purple border; others have an additional band of straw-color tissue that in turn is surrounded by a dark border. The striking difference in the leaf spots of the two varieties of Sudan grass is in the color because Tift Sudan

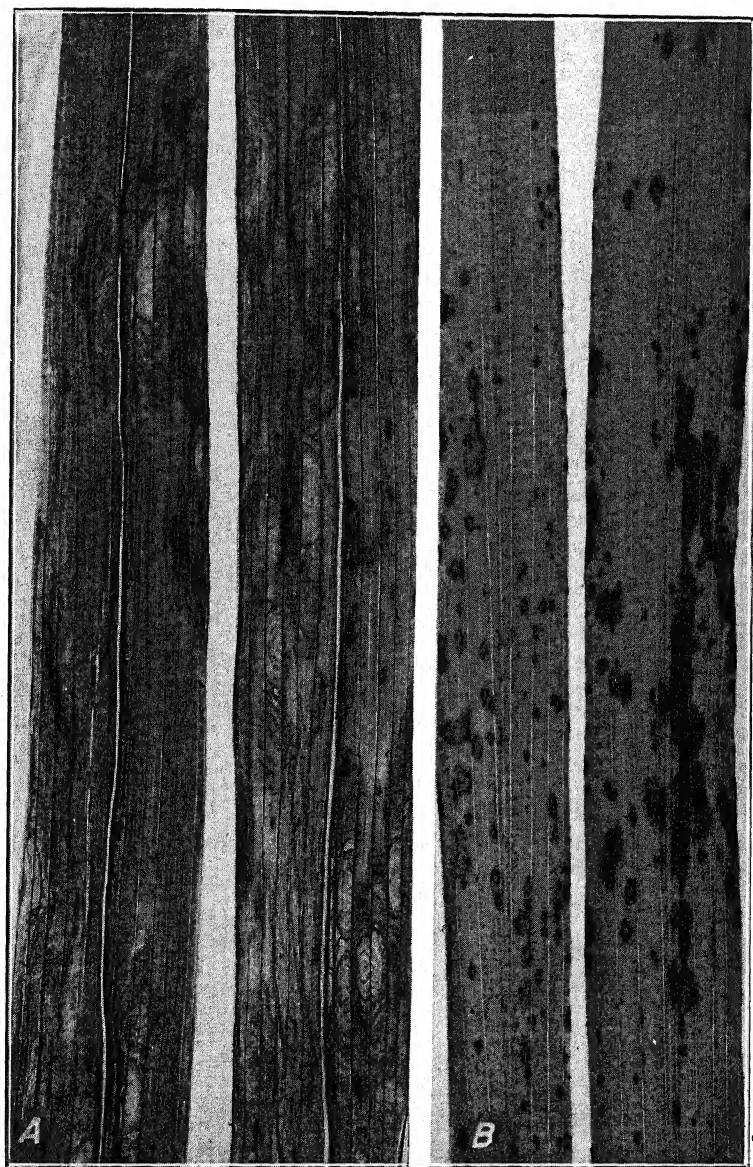


FIG. 1. Target-spot on Sudan grass.

grass has the tan pigment, hence, the tan leaf spot, whereas common Sudan grass contains the dark pigment, hence, the purplish-black lesions.

Under field conditions the fungus sporulates rather sparsely on both the upper and lower surfaces of infected leaves. Furthermore, sporulation is restricted to the dead, brown tissue of the lesions, and this paucity of fruiting may account for the slow spread of the disease. When infected leaves are placed in a moist chamber, however, sporulation is profuse, at first restricted to the lesions but later, as the leaf tissue becomes flaccid, the leaf surfaces may be completely covered with spores.

The conidia of the fungus are usually moderately curved, widest at or near the middle and taper slightly toward the rounded ends, regardless of the host from which they were collected (FIG. 2, A, B, C, D). Although the conidia of this *Sorghum* fungus are golden yellow to light olivaceous, resembling the conidia of *Cochliobolus heterostrophus* (*Helminthosporium maydis* Nisikado and Miyake) in color, they are less strongly curved, smaller, and have fewer septa than the corn fungus. The average number of septa in the conidia of the *Sorghum* fungus is 5.1, whereas in the corn fungus it is 7.7. The greater average number of septa in the conidia of the corn fungus is one of the more striking morphological characteristics that can be used to distinguish this fungus from the one here described on *Sorghum*.

The conidia are borne on conidiophores that usually emerge singly from a stoma, or directly through the epidermis (FIG. 2, D, a), but under favorable conditions two to four may emerge from a single stoma (FIG. 2, D, b). Comparatively few conidia seem to be borne on each conidiophore, there being often only one conidium under field conditions, whereas in a moist chamber two to four are often produced, as indicated by the number of geniculations (FIG. 2, D, a, b). In a moist chamber, or on agar where there is abundant moisture, a conidium borne on the primary conidiophore, and while still attached to it, may germinate and produce a secondary conidiophore bearing one or two conidia (FIG. 2, D, c). This may be repeated, the secondary spore germinating to produce a tertiary conidiophore, which in turn bears one or two conidia, giving the appearance of the conidia being

superimposed and produced in a chain-like manner. These secondary conidiophores are usually much shorter than the primary ones, but the secondary conidia may be as large as the primary ones. This type of conidial proliferation is very characteristic of this fungus and aids in distinguishing it from several other species of *Helminthosporium* that occur on grasses (4). In water or on agar, the conidium germinates usually by producing two polar germ tubes (FIG. 2, D, d), and oftentimes a germ tube-like hypha emerges from an attached conidiophore (FIG. 2, D, e).

The fungus grows well on corn-meal and potato-dextrose agars forming a compact mass of aerial hyphae and conidia. As the colony becomes older, sporulation becomes usually more abundant near the margin. On both corn-meal and potato-dextrose agar, the growth is whitish at first, turning light gray or light grayish-brown when the fungus begins to sporulate and later grayish-olive to slate olive (8). Small whitish upright tufts of hyphae are sometimes formed within or on the border of the mat. The growth on potato-dextrose agar is more profuse than that on corn-meal agar. When the fungus grows from diseased leaf fragments plated on potato-dextrose agar, the surrounding agar becomes a vinaceous-cinnamon or fawn color. On corn-meal agar the color is much fainter, being light vinaceous-cinnamon when distinguishable. In a solution of carrot decoction plus one per cent dextrose, the fungus grows well, forming a fluffy mat of mycelium on the surface of the liquid. The color of the mycelial mat is white at first and later grayish-olive to slate olive.

TAXONOMY OF THE FUNGUS

Helminthosporium sorghi was described by Schweinitz (10, p. 279) in 1832 from decaying leaves of *Sorghum* collected near Lititz, Pennsylvania. Later, Cooke (3, p. 141) used the same name for a fungus that he found on *Sorghum* that had been collected by Ravenel and sent to him for identification, which Ravenel issued later as No. 167 of his *Fungi Americani Exsiccati*. Saccardo (9, p. 420), seeing that the specific name had been pre-occupied, set up the new name *Helminthosporium cookei* Sacc. Hence, *H. sorghi* Cooke became a synonym of *H. cookei* Sacc.

The writers examined the type specimen of *Helminthosporium sorghi* Schw. from the Schweinitz Collections in the herbarium of the Academy of Natural Sciences of Philadelphia, and were unable to find conidia of a *Helminthosporium*. We also examined what undoubtedly is co-type material, in the Mycological Collections of the Bureau of Plant Industry and in the Farlow Herbarium, Harvard University, and again it was impossible to find conidia. There were, however, black setae of a *Colletotrichum* sp. in typical anthracnose lesions present in abundance on these leaf specimens. Whether Schweinitz observed these setae and mistook them for conidia of *Helminthosporium* cannot be determined now, although he described the *Helminthosporium* "pustules" as being black with conidia that were concolorous. In addition, the lesions on these leaf specimens do not resemble those caused by our fungus, so it seems obvious that the organism causing the leaf spot on *Sorghum* here described is clearly distinct.

We have also examined Ravenel's Fungi Americani Exsiccati specimen No. 167 and found a dark, crust-like, effuse fungus growth on the surface of a *Sorghum* culm. The fungus was superficial, produced no lesions, indicating the organism was probably a saprophyte. A few dark, thick-walled, straight conidia were found that looked wholly different from those produced by our fungus. These conidia were not associated with conidiophores that resemble those of the genus *Helminthosporium*.

Ciferri and Gonzalez Fragoso (2) described *Helminthosporium sudanensis* Frag. and Cif. on the flower parts of Sudan grass [*Sorghum vulgare* var. *sudanense* (Piper) Hitchc.]. Examination of specimens of the above material from the Farlow Herbarium and the Mycological Collections of the Bureau of Plant Industry reveals conidia that are mostly sharply curved, with the central cell larger and darker than the others, and the end cells practically hyaline. According to Boedijn (1), such fungi should be placed in the genus *Curvularia*. Since the fungus on Sudan grass described by Ciferri and Gonzalez Fragoso has, in addition to the above characteristics, conidia that are 4-septate and the conidial measurements agree with those of *C. geniculata* (Tracy and Earle) Boedijn, it is likely that species.

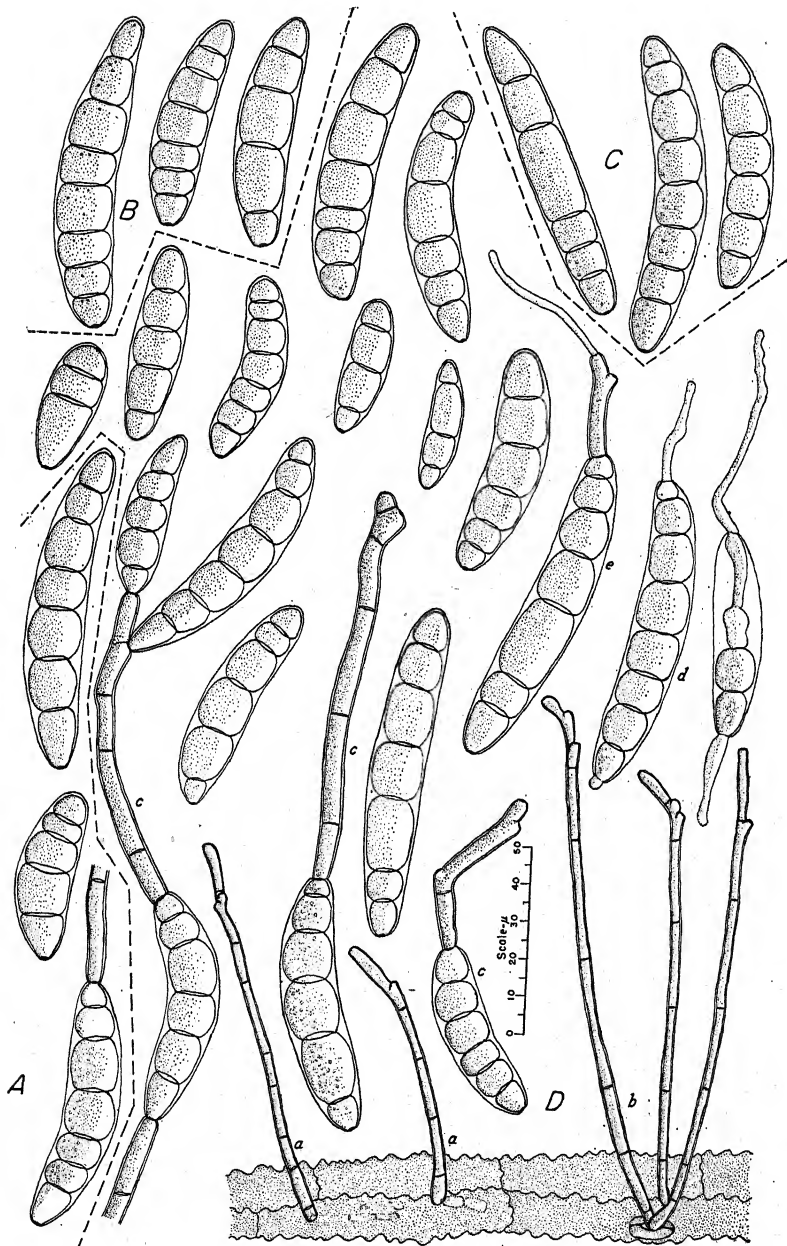


FIG. 2. Conidia and conidiophores of *Helminthosporium sorghicola*.

It appears, therefore, that the fungus that produces the target spot on *Sorghum* spp. described in this paper is widely different from the species of *Helminthosporium* previously reported on these hosts, and we believe it is an undescribed *Helminthosporium*. Since it occurs on several species of *Sorghum*, and because *sorghii* and *sudanensis* have already been used as specific names in the genus *Helminthosporium*, we propose to use the specific epithet *sorghicola* for the new species described hereafter.

***Helminthosporium sorghicola* sp. nov.**

Conidiophoris singulis vel 2-4-caespitosis, typice simplicibus interdum ramosis, olivaceo-brunneis, $5.5-10.5\ \mu$ in diam., $115-700\ \mu$ longis vel aliquando in madore longioribus; conidiis secundariis vulgaribus, brevioribus, $5-7\ \mu$ in diam., $25-200\ \mu$ longis; conidiis aureo-flavis usque pallide olivaceis, $20-105\ \mu \times 8.5-20.6\ \mu$, plerumque curvatis, circa medium latissimis et apices rotundatos versus attenuatis, 2-8-septatis, tenui-tunicatis, hilo inconspicuo praeditis.

Hab. in foliis glumisque *Sorghii* spp., U. S.

Producing well-defined spots on *Sorghum* spp., mostly in the Southern United States. Spots small, tan or reddish-purple, depending on host pigment—tan on Tift. Sudan grass, reddish-purple on common Sudan grass; older lesions usually show target or zonate pattern, with light centers, surrounded by narrow band of darker tissue, and this bordered by a wider band of light tissue, etc. Lesions range in size from barely visible to 1×15 mm.; these may coalesce to involve the whole leaf. Lesions at first round to elliptical, becoming more elongated as they are somewhat limited by the leaf veins. Fruiting of fungus on leaves and glumes sparse under field conditions, abundant when infected tissue is placed in moist chamber.

Conidiophores arising singly or in groups of 2 to 4, usually from stomata or occasionally singly between ordinary epidermal cells; typically simple, occasionally branching; dark, olivaceous brown; measuring 5.5 to $10.5\ \mu$ in diameter and 115 to $700\ \mu$ in length, sometimes longer under very moist conditions; secondary conidiophores common, especially under moist conditions, shorter, 5 to $7\ \mu$ in diameter and 25 to $200\ \mu$ in length. Conidia on Tift. Sudan grass leaves in moist chamber golden yellow to light olivaceous, measuring 20 to $105\ \mu \times 8.5$ to $20.6\ \mu$ (means = $59.2 \times 14.1\ \mu$), usually curved, widest near the middle, and tapering slightly toward rounded ends; 2 to 8 septa (mean = 5.1); peripheral wall thin; hilum moderately broad, not conspicuous; germinating by the production of two polar germ tubes.

Found on diseased leaves of *Sorghum vulgare* var. *sudanense* (Common) × *Sorghum vulgare* (Leoti) hybrid, Tifton, Ga., February 15, 1939; on leaves of *Sorghum vulgare* var. *sudanense* (Piper) Hitchc. (Tift variety), Gainesville, Fla., August 12, 1942; Tifton, Ga., August 19, 1943 (type), Herb. No. 896; on leaves of *Sorghum vulgare* Pers., Baton Rouge, La., July 1944, and on leaves of *Sorghum halepense* (L.) Pers., Cordele, Ga., August 22, 1945.

In addition to the above, *Helminthosporium sorghicola* has been found on the following specimens in the Mycological Collections of the Bureau of Plant Industry under the name *Helminthosporium sorghi* Schw.; on *Sorghum halepense*, College Station, Texas, fall, 1889, H. S. Jennings No. 80; on *Sorghum halepense*, College Station, Texas, January 10, 1890, H. S. Jennings; on *Sorghum halepense*, Austin, Texas, August, September, October, November, 1900, W. H. Long No. 447; on *Sorghum*, Etheridge, N. C., August 14, 1905, F. L. Stevens No. 404; on *Sorghum halepense*, San Antonio, Texas, July 29, 1910, W. P. C.

The writers wish to express their thanks to Dr. Francis W. Pennell, The Academy of Natural Sciences of Philadelphia, Mr. A. P. D. Piquet, Farlow Herbarium, Harvard University, and Mr. John A. Stevenson, Bureau of Plant Industry Station, for placing pertinent material at our disposal for study; to Miss Edith Cash for preparation of the Latin diagnosis; and to Dr. D. C. Bain, Louisiana State University, for sending a specimen of *Sorghum* M. N. 608.

Type specimens of *Helminthosporium sorghicola* have been deposited in the Mycological Collections of the Bureau of Plant Industry, Beltsville, Maryland, and in the Farlow Herbarium, Harvard University, Cambridge, Massachusetts.

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PLANT INDUSTRY STATION, BELTSVILLE, MD.

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EXPLANATION OF FIGURES

FIG. 1. *Helminthosporium sorghicola* on leaves of A. common Sudan × grass Leoti (*Sorghum*) hybrid; B. common Sudan grass, × 2.

FIG. 2. *Helminthosporium sorghicola*, drawn with the aid of a camera lucida, A, B, C and D × 500, except D, a, b × 250. A, Conidia from Johnson grass, *Sorghum halepense*; B, conidia from *Sorghum vulgare*, variety Rex; C, conidia from *Sorghum vulgare*, variety M. N. 608; and D, conidia and conidiophores from *Sorghum vulgare* var. *sudanense* (Tift); a, single conidiophores emerging directly through the leaf epidermis; b, group of conidiophores emerging through a stoma; c, primary conidia that have germinated and produced secondary conidiophores and conidia; d, germinating conidia, producing polar germ tubes; and e, primary conidium having produced a secondary conidiophore, a germ tube-like hypha now emerging from the distal end of this conidiophore.

TWO UNUSUAL FUNGI FROM GLACIER NATIONAL PARK, MONTANA¹

E. B. MAINS

(WITH 1 FIGURE)

In 1941, the writer spent several weeks in Glacier National Park, Montana. Among the fungi collected, two, *Mesopsora Hypericorum* and *Mitrula gracilis*, were of more than ordinary interest. Both were obtained on the continental divide along the trail from Logan Pass to Hidden Lake at altitudes around 7,000 feet.

MESOPSORA HYPERICORUM (Winter) Dietel

As far as the writer is aware this is the first report of *M. Hypericorum* for North America. The following description has been prepared from the collection.

Uredinia hypophyllous and caulicolous, golden to orange yellow when fresh, whitish when dried, pulverulent, flat to somewhat pulvinate, circular to elliptic in outline, 0.2–0.8 mm. wide, ruptured epidermis fairly conspicuous; urediniospores catenulate in short chains, variable in shape, broadly ellipsoid, obovoid to globoid, 18–24 μ long, 14–18 μ wide, wall colorless, 1.5–2.0 μ thick, moderately verrucose with irregular warts; telia not seen.

On *Hypericum Scouleri* Hook., Logan Pass, Trail near Hidden Lake, Glacier National Park, Montana, September 20, 1941, E. B. Mains (6121).

The rust was first described by DeCandolle as *Uredo Hypericorum*. Winter (16) apparently first described the telia and placed the species in *Melampsora* because the telia are typical of that genus. For a number of years the rust was treated as a *Melampsora* having only uredinia and telia. According to the Sydows (14), Gobi and Tranzschel were the first to note that the uredinia differed in several important aspects from the uredinia

¹ Paper from the Herbarium and the Department of Botany, University of Michigan.

of species of *Melampsora*. Instead of having pedicellate spores and paraphyses as in other species of *Melampsora* the sori lack paraphyses and the spores are catenulate in short chains. Fischer (5) also found the same. They concluded therefore that the sori are caeomoid aecia and that the species has aecia and telia and lacks uredinia, and therefore is a melampsoropsis. In support of this interpretation Fischer states that intercalary cells occur in the spore-chain and the sorus is bordered by a slight layer of thin-walled cells suggesting a peridium. In the Montana collection it has not been possible to determine whether intercalary cells occur. Sections through the sori show a slight layer of large thin-walled cells at the margin sometimes extending a short distance upward under the ruptured epidermis. It is questionable that a peridium is formed.

Klebahn (9) found uredinia having paraphyses on *Hypericum humifusum* and concluded that they belonged to *M. Hypericorum* and that therefore the species is an autoeumelampsora. However, the uredinia on *H. humifusum* were not associated with any other spore-form and although on the other collections the sori designated as "caeoma" were found with telia the "uredo" sori did not occur. He reports an experiment in which somewhat scanty telial material giving weak germination was sown on several species of *Hypericum*, *Abies pectinata*, *Picea excelsa* and *Larix decidua* without results. He expressed considerable doubt concerning his conclusions and in 1914 he (10) described the rust on *H. humifusum* as a new species, *Uredo* (*Melampsora*?) *hyperici-humifusi*, and treated *Melampsora Hypericorum* as a melampsoropsis. The Sydows (14, 15) in their monograph of the rusts have followed this latter treatment.

Dietel (2) has decided that the sori with catenulate spores are uredinia. He points out that they have never been reported associated with pycnia and that they are produced in successive generations during the summer. Also they are similar to the uredinia of species of *Coleosporium* and *Chrysomyxa* which produce aecia on conifers. He concludes that the rust is probably heteroecious with aecia on conifers and with uredinia and telia on species of *Hypericum* and that it belongs in a genus intermediate between

Melampsora and *Coleosporium* for which he proposes the name *Mesopsora*.

The correct interpretation of the sori of this rust and its relationship to other species of the Melampsoraceae cannot be determined with certainty until its life history is worked out through cultures. Until such is done it seems best to follow Dietel's treatment. The absence of pycnia strongly indicates that the sori are uredinial in function. If the rust is an autoecious melampsoropsis as suggested by the Sydows and Fischer it would be necessary to assume that only secondary or repeating aecia (aecidioid uredinia according to Arthur's terminology) have been collected since pycnia have not been reported. Such a species would be unique in *Melampsora*. Since the rust appears to be fairly common in Europe, primary aecia accompanied by pycnia would be expected in some collections. If the rust is heteroecious as suggested by Dietel pycnia would not be expected on *Hypericum*. Also the negative results obtained by Klebahn in cultures of germinating teliospores on species of *Hypericum* would be expected. Since intercalary cells are known to occur in the uredinia of *Coleosporium* (Moss, 11) their presence as noted by Fischer in the uredinia of *Mesopsora Hypericorum* might be expected.

The Sydows have reported *M. Hypericorum* from Europe, Asia Minor and Africa. It also has been reported from Australia and Japan but such reports may be based on misdeterminations of *Melampsora Kusanoi* Diet. As far as I am aware *Mesopsora Hypericorum* has not been previously reported for North America. Therefore the isolated occurrence of the species on an indigenous species of *Hypericum* is an interesting instance of disjunct distribution.

MITRULA GRACILIS Karsten (FIG. 1, A-E)

In his monograph of the Geoglossaceae of North America Durand (3) includes two species of *Mitrula* on mosses, *M. gracilis* Karsten and *M. muscicola* E. Henn. Only a few localities are given for each. *M. gracilis* is listed for Labrador and Newfoundland "attached to and evidently parasitic on *Paludella squarrosa*." It is stated that Rostrup also has reported the species from Greenland. Apparently the only report of the species for the United

States is that of Seaver (13) who found it "on some species of bog moss in Geneva Creek Canyon, Colorado, at an elevation above 8000 ft." Only one collection of *M. muscicola* was reported by Durand in his monograph "on mossy stems (*Webera nutans*) . . . at about 7000 feet elevation, Laggan, Alberta." In 1915 Durand (4) also collected it "on wet moss at the water's edge, Lake Agnes, Alberta." In the United States it is known only from collections made by Kauffman (8) at Tolland, Colorado, at 9,500 feet and Leal, Colorado, at 8,600 feet. The two species are therefore rare for North America. Durand (4) states that he searched for them carefully but in vain at various points along the Alaskan coasts as far north as Skagway. In the United States only the three collections from Colorado have been made. The writer has sought for them for a number of years without success. However, in 1940 a *Mitrula* was found in moss around the margins of pools near the Hidden Lake Trail a short distance from Logan Pass. The fungus probably was parasitic since it was fruiting in patches of dead moss. The following description has been prepared.

Ascomata capitate, very variable, 10-30 mm. long, the ascogenous portion irregularly globoid, obovoid, ovoid, ellipsoid, reniform, flattened globoid, or occasionally almost cylindric or spathulate, $2-6 \times 1.5-7$ mm., smooth, convoluted, somewhat cerebriform or ridged when fresh, the ridges and convolutions more pronounced when dry, light orange-yellow to orange-buff (Ridgway) when fresh, darker when dried, cinnamon-rufous, the stipe slender, 1 mm. wide, equal or somewhat wider above, concolorous with the head or somewhat lighter, smooth when fresh, longitudinally furrowed when dried; asci clavate, $60-74 \times 6-7 \mu$, pore staining blue with iodine, the ascospores fusoid-cylindric, $9-12 \times 2-2.5 \mu$, oblique or biseriate, the paraphyses filiform, slightly wider above, $1.5-2 \mu$, as long as the asci.

Growing in and apparently killing moss, *Aulacomnium palustre* (Web. & Mohr.) Schwaegr. around margins of pools, Trail to Hidden Lake, Logan Pass, Glacier National Park, Montana, 8-20-41, E. B. Mains (6117).

The Montana collection is similar to those reported by Kauffman (8) as *M. muscicola* from Colorado. Since he did not give a description the following has been prepared from his collections in the Herbarium of the University of Michigan and from his notes.

Ascomata capitate, 10–20 mm. long, the ascogenous portion obovoid to subglobose, 3–5 × 3–4 mm., pale ochraceous, darker when dried, even, rugose or lacunose-wrinkled, sharply differentiated from the stipe, the stipe slender, even, creamy-whitish, subpellucid, elastic, glabrous when fresh, yellowish brown and longitudinally furrowed when dried; asci clavate, 65–90 × 6–9 μ , the pore staining blue with iodine, the ascospores fusoid-cylindric, 10–14 × 2 μ , biseriate, the paraphyses filiform, slightly thickened above.

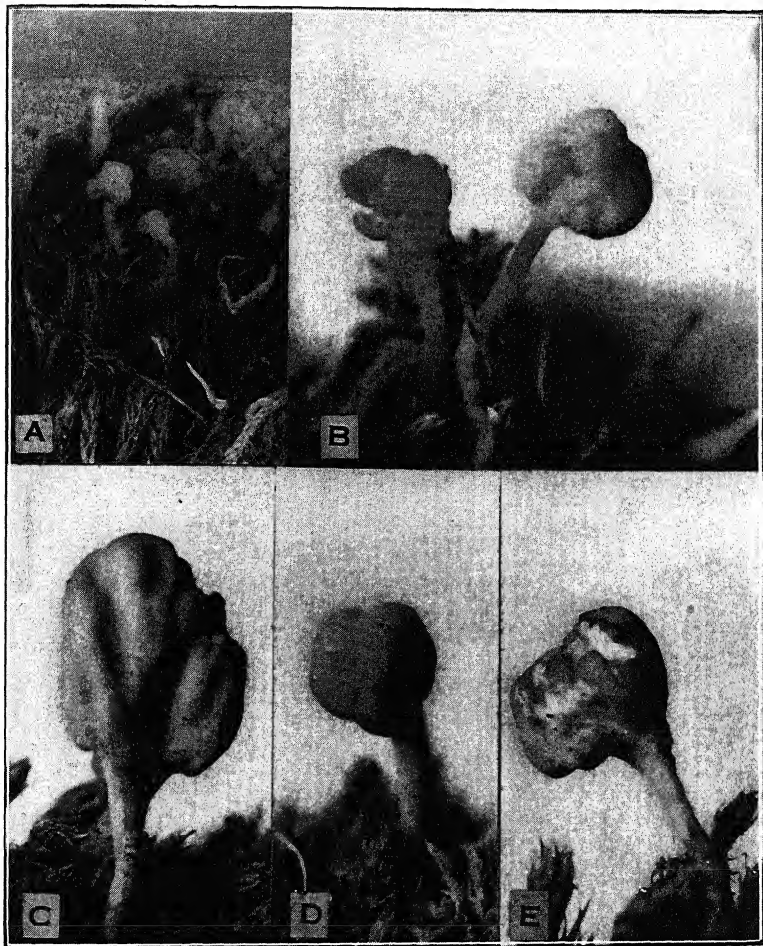


FIG. 1. *Mitrula gracilis* from Glacier National Park, Montana. A. Ascomata arising from moss, approximately 1 \times ; B–E. Ascomata showing some of the variations in shape and surface, 5 \times .

Growing on living moss in a swampy ground, Leal, Colorado (8,600 ft.), Aug. 11, 1917, C. H. Kauffman; on mossy stump, Tolland, Colorado (9,500 ft.), Sept. 14, 1920, C. H. Kauffman.

According to Kauffman's notes the stipes of his collections were much lighter in color than those of the Montana collection. When dried they show little if any difference.

Mitrula gracilis and *M. muscicola* do not differ greatly according to the descriptions of Karsten (7) and Henning (6). In treatments where the species are separately maintained the principal differences noted are that *M. gracilis* has an orange-brown more or less even head and a lighter colored stipe whereas *M. muscicola* has a more convoluted, cinnamon-brown head and concolorous stipe. Such variations may occur within a collection, as in the Montana collection. Although Durand treated them as separate species he did so doubtfully. Seaver also has expressed considerable doubt concerning their separation. Nannfeldt (12) in his recent article concerning the Geoglossaceae of Sweden has concluded that they are synonymous. With this the writer agrees. *M. gracilis* appears to have been more frequently collected in Europe than in North America, although it is far from common. Its arctic and high alpine distribution probably accounts to some extent for the few collections.

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SIX NEW INDIAN DISCOMYCETES

EDITH K. CASH

Among specimens of Discomycetes collected by Sultan Ahmad in various localities in India and Pakistan and sent to the writer for examination during the past few months, a specimen of *Dasyscyphella*, two of *Humaria*, and three of *Humarina* appear to differ from any species of these genera that could be found in the literature and are therefore named here as new. Type specimens of these fungi are deposited in the Mycological Collections of the Bureau of Plant Industry, Beltsville, Maryland, and duplicate material of some of them in the herbarium of the collector at Lahore, Pakistan.

1. *Dasyscyphella indica* sp. n.

Apothecia substipitata, ceracea, turbinata, flava, 1-2 mm. diam., margine et extus e pilis echinulatis, hyalinis, $40-50 \times 3-4 \mu$ albopruinosa; asci cylindrici, apicibus attenuatis, octospori, $100-120 \times 7-8 \mu$; ascospores hyalinae, anguste clavatae, multiguttulatae, $35-45 \times 2 \mu$; paraphyses tenues, usque 2μ inflatae; excipulum hyalinum, plectenchymaticum.

Apothecia substipitate, waxy, turbinate, 1-2 mm. in diameter, hymenium plane, Capucine yellow,¹ ferruginous when dry, exterior concolorous, white-pruinose, margin fimbriate; asci cylindrical, narrowed and with wall thickened at the tips, gradually attenuated to a short pedicel, eight-spored, $100-120 \times 7-8 \mu$; ascospores hyaline, narrow-clavate, multiguttulate, acute at the lower end, often arcuate or sigmoid when free from the ascus, $35-45 \times 2 \mu$; paraphyses numerous, delicate, only slightly enlarged at the tips up to 2μ ; exciple hyaline, plectenchymatous, hairs echinulate, hyaline or subhyaline, $40-50 \times 3-4 \mu$.

On mossy bark of trees, Mussoorie, July 20, 1940, S. Ahmad 434.

D. indica differs from *Erinella corticola* Mass., described from India (Kew Bull. 1898, p. 115, 1898), in the flexuous, instead of fusoid paraphyses, and from several other somewhat similar species of *Erinella* in the dimensions of the asci and spores.

¹ Except where noted, color readings are from Ridgway, R. Color standards and nomenclature, Washington, D. C., 1912.

2. *Humaria ahmadii* sp. n.²

Apothecia sessilia, carnosa, 1-3 mm. diam., rubra, patelliformia; asci cylindrici, octospori, ad apices rotundati, $180-190 \times 18-20 \mu$; ascosporae hyalinae, late ellipsoideae, uniseriatae, verrucosae, 2-guttulatae, saepe solum quatuor maturascentia, $15-20 \times 9-12 \mu$; paraphyses flexuosae, numerosae, ad apices usque $5-6 \mu$ incrassatae; setae paucae, pallide brunneae, subacutae, simplices vel 1-2-septatae, $75-100 \times 8-11 \mu$.

Apothecia sessile, fleshy, 1-3 mm. in diameter, bright red,³ fading to pale olive gray or nearly white in dried specimens, patelliform, margin nearly even, excipular hairs short, inconspicuous; asci cylindrical, rounded at the apex, gradually attenuated toward the base, eight-spored, $180-190 \times 18-20 \mu$; ascospores hyaline, broadly elliptical, uniseriate, coarsely verrucose, two-guttulate, usually only four becoming mature, $15-20 \times 9-12 \mu$; paraphyses numerous, flexuous, unbranched, enlarged at the tips to $5-6 \mu$; setae inconspicuous, pale brown, subacute, simple or 1-2-septate, $75-110 \times 8-11 \mu$.

On the ground, Lahore, Pakistan, Nov. 24, 1947, S. Ahmad 2226.

The very short, inconspicuous setae constitute the most distinctive character of this species. It appears to be close to *Cheilymenia calvescens* Boud., from which it differs in the shorter setae and coarsely verrucose, rather than minutely echinulate spores.

3. *Humaria pallidisetosa* sp. n.

Apothecia patelliformia, carnosa, 6-8 mm. diam., avellanea, margine denticulato; asci cylindrici, apicibus subobtusis, octospori, $200-250 \times 13-15 \mu$; ascosporae uniseriatae, hyalinae, late ellipsoideae, glabrae, $15-18 \times 9-11 \mu$; paraphyses numerosae, granulosa, ad apices $3-4 \mu$; setae pallidissime brunneae vel subhyalinae, flexuosae, angustae, usque $250-300 \times 5-8 \mu$, glabrae vel minute echinulatae, septatae.

Apothecia patelliform, soft fleshy, 6-8 mm. in diameter, wood brown to drab, drying cinnamon drab, margin minutely denticulate; asci cylindrical, slightly flattened at the tips and narrowed below, eight-spored, $200-250 \times 13-15 \mu$; ascospores uniseriate, hyaline, broadly ellipsoid, smooth, $15-18 \times 9-11 \mu$; paraphyses numerous, granulate, $3-4 \mu$ at the apices; setae very pale brownish to subhyaline, flexuous, narrow, smooth to finely echinulate, septate, narrowed and rounded at the tips, $250-300 \times 5-8 \mu$.

² As pointed out by Kanouse (Mycologia 39: 655. 1947), *Humaria* is the valid generic name under the International Rules of Nomenclature for the species generally known as *Lachnea* or *Patella*.

³ Ridgway color reading not made on fresh material.

On the ground, Rohtak, Punjab, Jan. 17, 1947, S. Ahmad 1776.

The fungus is close to *Patella gilva* (Boud.) Seaver in dimensions, but differs from the latter in the nearly hyaline setae and in the color of the hymenium.

4. *Humarina plumbeo-atra* sp. n.

Apothecia cupuliformia, sessilia vel partim immersa, carnosa, glabra, margine leniter crenata, 1.5–2 mm. diam., violaceo-ardosiaca usque plumbeo-atra; asci cylindrici apicibus obtusis, octospori, $130\text{--}175 \times 12\text{--}15 \mu$; ascospores uniseriatae, late ellipsoideae, glabrae, utrinque guttulate, $13\text{--}14 \times 7\text{--}9 \mu$; paraphyses tenues, ascos superantes, apicibus $2.5\text{--}3.5 \mu$ crassis; excipulum ex hyphis tenuibus plectenchymatice intertextis, extus cellulis angulosis $10\text{--}15 \mu$ diam. compositum.

Apothecia sessile or partly immersed in soil, fleshy, smooth, margin slightly crenate, 1.5–2 mm. in diameter, hymenium and exterior dark violet slate to plumbeous black, flesh purple when crushed; asci cylindrical, obtuse at the tips, gradually attenuated below, eight-spored, $130\text{--}175 \times 12\text{--}15 \mu$; ascospores uniseriate, broadly ellipsoid, smooth, with one small guttule at each end, $13\text{--}14 \times 7\text{--}9 \mu$; paraphyses slender, longer than the asci, gradually enlarged to $2.5\text{--}3.5 \mu$ at the tips; exciple composed of fine plectenchyma within, the outer layer of thin-walled, subglobose to angular cells $10\text{--}15 \mu$ in diameter.

On the ground beside a water course, Ladhar, Sheikhpura, Punjab, July 8, 1946, S. Ahmad 1640.

Among the species nearest in color of the hymenium, *Humarina purpurea* Seaver has rough spores, and both *Humaria plumbea* Fr. and *Peziza violacea* Pers. ex Fr. have much larger apothecia.

5. *Humarina umbrina* sp. n.

Apothecia patelliformia usque plana, aliquantus plicata, carnosa, umbrina, 7–8 mm. diam., extus glabra vel minute pustulata, margine subundulato; asci cylindrici, apicibus obtusis, octospori, $200\text{--}250 \times 11 \mu$; ascospores uniseriatae, hyalinae, ellipsoideae, glabrae, $12\text{--}15 \times 7\text{--}9 \mu$; paraphyses flexuosae, apicibus usque $4\text{--}5 \mu$ incrassatis et dense granulosis; excipulum e cellulis tenuibus, subglobosis usque 25μ diam. compositum.

Apothecia patelliform to applanate, sessile, more or less plicate, fleshy, 7–8 mm. in diameter, hymenium Saccardo's umber, drying sepia, exterior concolorous, smooth to minutely pustulate, margin slightly undulate; asci cylindrical, obtuse at the tips, narrowed below, eight-spored, $200\text{--}250 \times 11 \mu$; ascospores uniseriate, hyaline,

ellipsoid, smooth, $12-15 \times 7-9 \mu$; paraphyses flexuous, unbranched, tips swollen to $4-5 \mu$ and filled with fine yellowish granules; exciple of thin-walled hyaline subglobose cells, 25μ in diameter.

On the ground, Lahore, Pakistan, Nov. 24, 1947, S. Ahmad 2225.

H. umbrina may be distinguished from *Peziza saccardiana* Cke. by the smooth spores. In *P. sepiatra* Cke. and *P. sepiatrella* Sacc., two species also similar in color, the spores are decidedly longer.

6. *Humarina zizyphi* sp. n.

Apothecia obconica usque turbinata, substipitata, 1-2 mm. diam., margine subundulato, sicco involuto, armeniacco-lutea usque ochraceo-aurantia, sicca daucino-ochracea; asci cylindrici apicibus deplanatis, longe pedicellati, octospori, $100-150 \times 13-15 \mu$; ascospores uniseriatae, hyalinae, glabrae, oblongo-ellipsoideae, guttulis parvis numerosis impletae, $20-25 \times 10-11 \mu$; paraphyses abundantes, hyalinae, granulosaе, ad apices $2-3 \mu$ inflatae, circa 50μ infra apices ramosae; excipulum subhyalinum e cellulis irregulariter angulosis $15-25 \mu$ in diam. compositum.

Apothecia obconic to turbinate, substipitate, 1-2 mm. in diam., margin slightly undulate and inrolled when dry, hymenium apricot-buff to ochraceous orange, carrot red to ochraceous tawny when dry, exterior concolorous, smooth; asci cylindrical, flattened at the tips, long pedicellate, $100-150 \times 13-15 \mu$; ascospores hyaline, smooth, oblong-ellipsoid, filled with many small oil globules, $20-25 \times 11-12 \mu$; paraphyses abundant, hyaline, granulose, branched about 50μ below the tips, slightly swollen to $2-3 \mu$ at the tips; exciple subhyaline, composed of irregularly angular cells $15-25 \mu$ in diameter.

On stones and on dead branches of *Zizyphus jujuba*, Ladhar, Sheikhpura, Punjab, Sept. 23, 1941, S. Ahmad 419.

In the form and size of the spores this fungus resembles *Humarina waterstonii* Seaver described from Bermuda on seeds of *Livistona chinensis* (Mycologia 31: 533. 1939), but differs from that species in the host plant and in the paler hymenium.

U. S. PLANT INDUSTRY STATION,
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A STUDY OF THE TEMPERATURE AND HUMIDITY REQUIREMENTS OF *ASPERGILLUS NIGER*¹

JOHN TYLER BONNER

(WITH 3 FIGURES)

There are a number of reasons why the temperature and humidity relations of fungi are important. First they are of physiological interest for they affect and limit the fundamental processes of growth and development. Also, of a more practical nature, they both provide information helpful in the problem of fungus or mildew control, and they are useful in laboratory tests of vulnerability or resistance of items to fungus attack, where the optimum conditions of temperature and moisture must be known.

Aspergillus niger was chosen as the organism for this study both because of its widespread occurrence and because of its frequent use as a test organism. (The latter is especially true of the strain chosen: United States Department of Agriculture No. Tc 215-4247.)

MATERIALS AND METHODS

The technique used was basically that of Galloway (1935). Briefly, small squares of cellophane streaked with fungus spores were suspended on threads over solutions of different concentrations of calcium chloride, giving atmospheres of different relative humidities. Since details of the methods play an important role in the reproducibility of results, they will be described here.

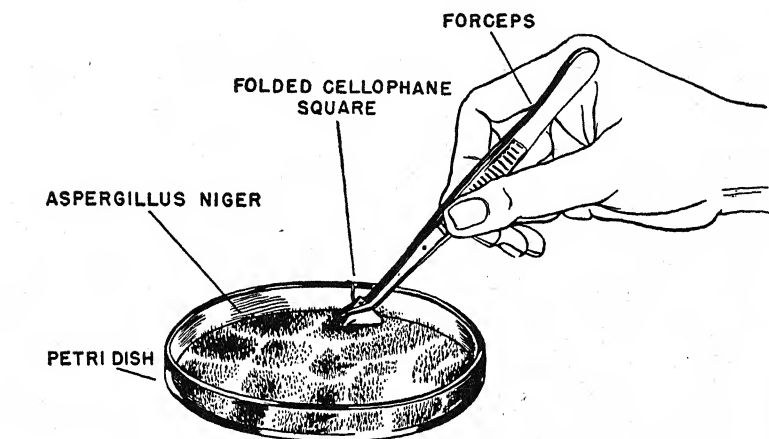
Aspergillus niger was grown in Petri dishes on the following media: Dextrose, 30 gm.; NaNO₃, 30 gm.; K₂HPO₄, 1.0 gm.; MgSO₄, 0.25 gm.; KCl, 0.25 gm.; agar, 15 gm.; distilled water,

¹ This study was carried out during the war as part of a tropical deterioration research program at the Aero-Medical Laboratory, Wright Field, Dayton, Ohio.

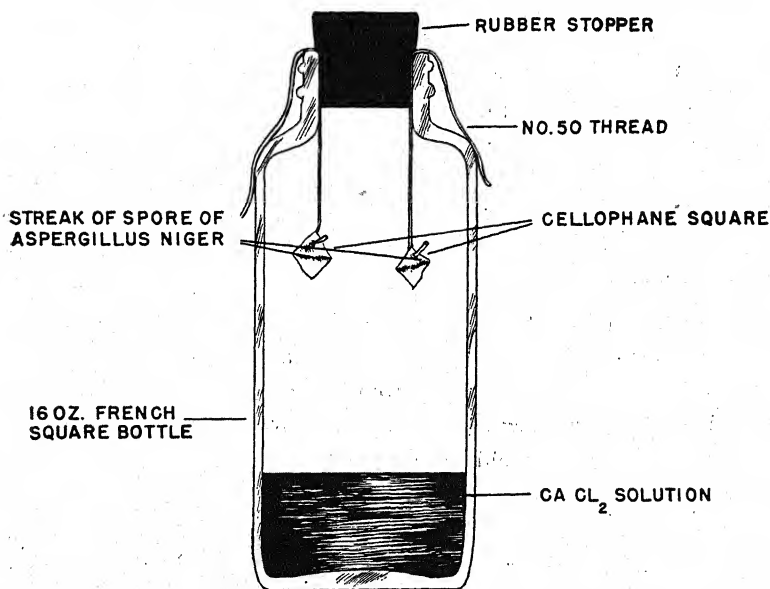
The author wishes to express his gratitude for the invaluable technical assistance of Mrs. Roberta Dingwall.

1000 ml. (pH adjusted to 6.8). The spore load was obtained from cultures that had grown at room temperature from seven to fourteen days.

The squares, onto which the spores were placed, were made



METHOD OF PLACING SPORES ON THE CELLOPHANE SQUARE



METHOD OF PLACING CELLOPHANE SQUARES IN TEST CHAMBERS

FIG. 1. Diagram illustrating the technique used.

from strips of cellophane² which had been immersed in five per cent malt extract (Difco) solution for ten minutes, dried in room air, and cut into squares 1 × 1 cm. Each square was strung onto a piece of number fifty white cotton thread and held by a simple knot. They were not sterilized because the germinating spores could always be identified as *Aspergillus niger* under the microscope. In the few instances where contaminants appeared, the squares were discarded.

To place a streak of spores on a square, the cellophane square was folded back so that two corners met and the fold was brought in contact with the surface of the fungus culture. This fold,

TABLE 1
CONCENTRATION OF CALCIUM CHLORIDE SOLUTIONS (AS DETERMINED BY
THE HARVEY (1910) METHOD) AND THE ESTIMATED RELATIVE
HUMIDITY OVER THESE SOLUTIONS (OBTAINED FROM
DATA OF THE DOW CHEMICAL COMPANY)

Concentration of Solution	Estimated Relative Humidity
26.5%	70%
21.9%	78%
17.1%	85%
10.0%	93%

studded with spores, was then rubbed gently against a piece of filter paper to even their distribution. The inoculated squares were then suspended over various concentrations of calcium chloride solution in 16 oz. French square bottles so that there were 4 squares in each bottle (FIG. 1).

Four different concentrations of calcium chloride solutions were prepared and each solution was divided so that 30 ml. were dispensed in a number of the 16 oz. French square bottles. The concentrations of the solutions were carefully checked by the Harvey (1910) technique. From information generously supplied by the Dow Chemical Company (1945) it was known what relative humidities could be expected over such solutions. This information is recorded in table 1.

The bottles containing the squares were placed in an incubator in which a fan had been installed to ensure constant temperatures

² The cellophane used was Du Pont PUT-O (30 gms. per square meter) which has received no special surface coating treatment of any sort, but is merely pure, regenerated cellulose.

and to prevent any temperature gradients. The length of time for the temperature inside a French square bottle (kept at room temperature, 24° C.) to reach equilibrium with the oven temperature was determined. By use of thermocouples it was found that at the near extreme incubator temperature of 45° C., in one-half hour the temperature inside the bottle was 1° C. low, and in one hour it had reached the incubator temperature.

The technique for making the germination observations was to remove a square from the bottle and fix the spores on the cellophane for staining by holding the surface containing the spores over steam rising from boiling water for about one-half to one minute. The square was then immersed in Linder's Lacto-phenol-cotton-blue, which was used as a mounting and staining fluid, and heated gently on a slide, under a cover-slip, to flatten out the cellophane.

The germination times were established by making stained preparations of replicates of any one experiment at different intervals of time approaching the expected germination time as determined by preliminary tests. The four sets of slides of each determination were carefully examined microscopically and an estimated germination time was established. The times are expressed $a \pm b$. The a indicates the most probable time; $\pm b$ the range of time within which germination certainly occurs (TABLE 2).

In this study "germination" is defined as that stage of development where the sides of the germ tube are first parallel, and "germination time" is defined here as the length of time required for at least ten spores to germinate on a cellophane square. This is contrary to the procedure used by many (see, for instance, Wellman and McCallan, 1942) where the per cent germination is plotted against time, and "germination time" is arbitrarily chosen as the time when fifty per cent, or some other per cent, germination has occurred. The reason for not using such a method is twofold: (1) it is difficult to get an even distribution of spores on the cellophane squares when using Galloway's (1935) technique, which is necessary to establish per cent germination, but more important, (2) from the practical point of view it is of greater value to know when the first spore germination will occur than to know the average time for all spores. This point was clearly shown, when in some

instances the per cent germination remained extremely low, but the few that did germinate grew and sporulated. (For example see figure 3, F.)

RESULTS

The germination time of *Aspergillus niger* USDA No. Tc 215-4247 was determined at 10°, 20°, 30°, 40°, 45°, 50° C. and at the following relative humidities for each of those temperatures: 100%, 93%, 85%, 78%, 70%. Most determinations were checked in at

TABLE 2
GERMINATION TIME OF *Aspergillus niger* AT DIFFERENT
TEMPERATURES AND RELATIVE HUMIDITIES

Temperature in °C.	Relative Humidity	Estimated Germination Time in Hours	Number of Experiments Performed
10	100	>100	1
20	100	12 ±1	4
20	93	12 ±1	4
20	85	17 ±3	4
20	78	>100	1
30	100	4 ±1	4
30	93	5.5 ±1	4
30	85	9 ±1	4
30	78	48 ±3	4
30	70	>100	1
40	100	5.5 ± .5	4
40	93	3.5 ± .5	4
40	85	8 ±2	4
40	78	33 ±3	4
40	70	>100	1
45	100	17 ±5	2
45	93	7 ±2	4
45	85	9 ±2	4
45	78	50 ±5	2
45	70	>100	1
50	100	>100	1

least four separate experiments. This checking was found necessary after preliminary experiments which showed that the variability of results was large. However, in a few instances the results were sufficiently definite to warrant fewer checks. The data from these experiments are recorded in table 2.

In order to appreciate fully the results given in table 2 they must be displayed in the form of a graph, but since there are three variables, ideally a three-dimensional graph is required. Taking heart from the ecologists (see Shelford, 1929) who have been confronted with the same problem, a two-dimensional graph has been

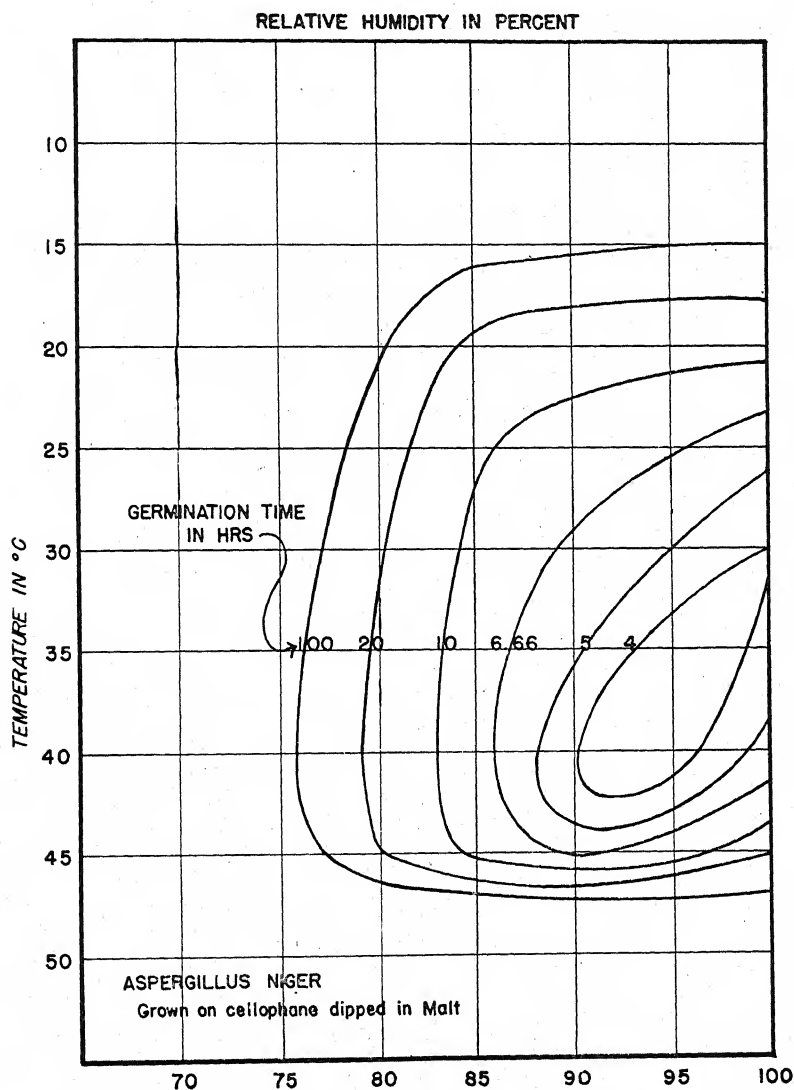


FIG. 2. Germination curves.

drawn (FIG. 2) in which the abscissa is relative humidity, the ordinate temperature, and "germination time" is represented by a family of curves.

The salient characteristics in relation to temperature and humidity that can be observed for *Aspergillus niger* in figure 2 are primarily the following: the optimum conditions for growth are at a relative humidity near 93 per cent, and at a temperature near 40° C; at 100 per cent relative humidity the optimum temperature is near 30° C. Thus we find that the optimum condition for growth for this species is not at saturation and that the optimum temperature varies with different humidities.

It should be clearly understood, when interpreting the graph in figure 2, that for two reasons the accuracy of the curve is limited: (1) as stated above the points on the graph are the "estimated" germination time within a range of time where it is certain germination occurred. Although these "estimated" points are believed to be fairly accurate, they are to a limited extent arrived at by human judgment and not entirely by objective measurement. (2) There are relatively few points on the graph and therefore there has been a considerable amount of interpolation and extrapolation and the possible error is multiplied by the fact that it is in three dimensions.

During the course of these experiments it was noted that the different conditions imposed on the germinating spores of *Aspergillus niger* definitely affected its morphology. It was especially true that under extreme conditions abnormalities in pattern occurred. These abnormalities were of two types. One occurred at high temperature, 45° C., and at high humidities, 100 per cent and 93 per cent, where the spores were greatly swollen (FIG. 3, A): Under optimal conditions slight swelling occurred (FIG. 3, B), but in these extreme cases the spore swelled to over ten times its normal volume. This swelling in no way prevented germination, as evidenced in figure 3, A. At a higher temperature, 50° C., where no germination occurs, there was no evidence of any swelling, in fact the spores showed signs of degeneration. The second type of abnormality appeared at low relative humidities, 70-78 per cent at all viable temperatures. In these instances the spore appeared to be of normal diameter, but the germ tube was enlarged (FIG.

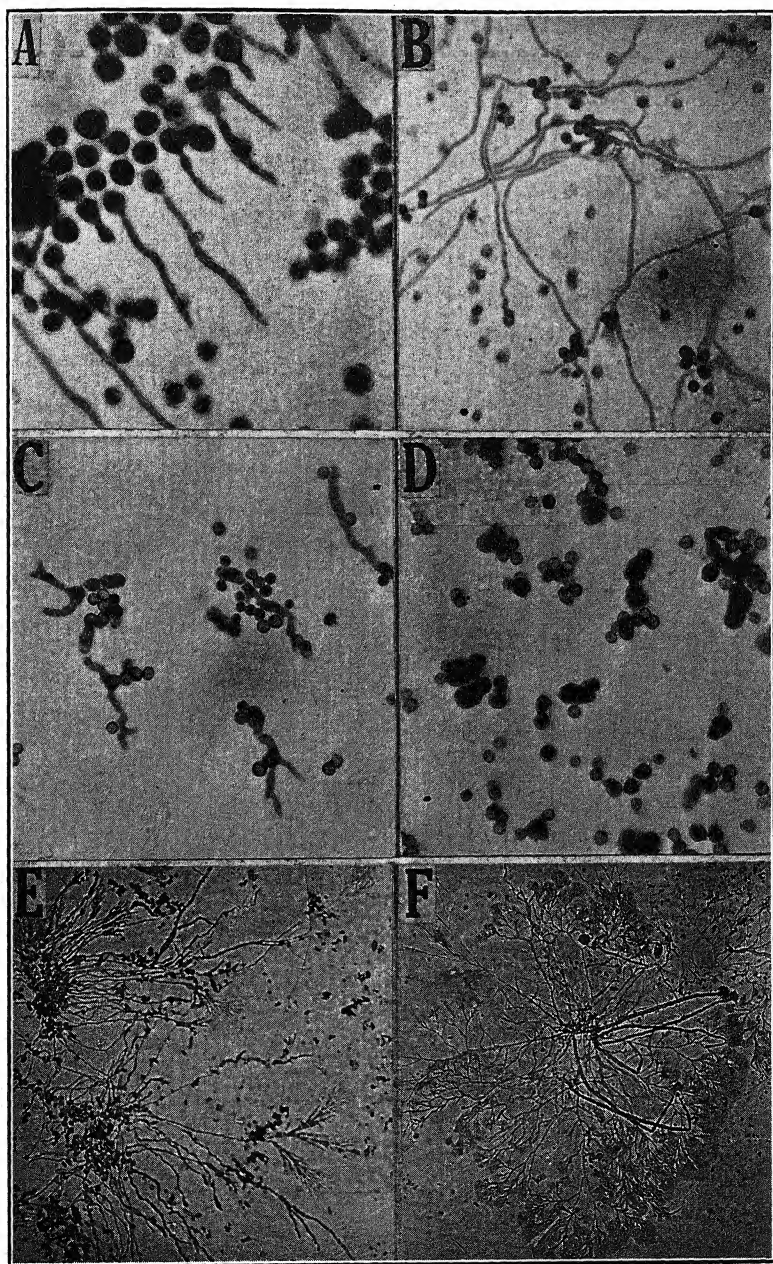


FIG. 3. Germinating spores.

3, C), sometimes even spherical (FIG. 3, D), rather than the normal thread-like construction. If the experiment was allowed to run for extended periods of time, often different types of peculiar segmented, flat, fanlike hyphal clusters resulted (FIG. 3, E, F). This apparently does not inhibit sporulation, as seen in figure 3, F where a sporangiophore rises from the center of the abnormal mycelium.

DISCUSSION

A comparison has been made between the results obtained here on *Aspergillus niger* with those to be found in the literature. Very little complete work has been done on the temperature and humidity requirements of any one fungus. Although Groom and Paniset (1933) give some data, the only work I have found that is sufficiently parallel to this study so that comparisons can be fruitfully made is that of Tomkins (1929).

Tomkins made detailed studies of two fungi: *Alternaria citri* and *Trichoderma lignorum*. His results, which are essentially similar for both of these fungi, differ in three major respects from *Aspergillus niger*: (1) The optimum relative humidity for Tomkins' fungi is 100 per cent, whereas for *Aspergillus niger* it is nearer 93 per cent. (2) In his fungi the optimum temperature is the same for all relative humidities, which is not true for *Aspergillus niger*. (3) In his fungi the temperature minimum becomes lower the higher the relative humidity, whereas in *Aspergillus niger* the temperature minimum is approximately constant between relative humidities of 85 to 100 per cent.

The reason for these major differences could be found in a number of directions. Tomkins' technique is very different—for instance he measures growth rate instead of germination time. Furthermore his actual testing conditions differ radically from those used here.

But also it is quite conceivable that different fungi vary in their response to temperature and humidity. To examine this point a survey was made of the minimum, optimum and maximum temperatures and humidities of numerous fungi. Briefly the following information on temperatures was obtained:

In a fairly representative group of mildews the range of minimum temperature for forty-four fungi is from -6° C. to 15° C., the range of optimum temperatures for fifty-one fungi is from 10° C. to 45° C., and the range of maximum temperatures for thirty-two fungi is from 25° C. to 51° C.

Similar data on humidities show that the minimum relative humidity for fifty-eight fungi is from approximately 70 per cent to approximately 98 per cent. Furthermore, the frequency distribution of minimum relative humidities for the fifty-eight fungi is fairly even between the limits of the range.

We have then ample evidence that fungi differ greatly in their response to temperature and humidity.

Unfortunately the basic problem of how temperature and humidity affect the processes of growth and development in any fungus, and what it is in different fungi that is affected so specifically by these environmental conditions, remains completely obscure. If anything is indicated it is that the relationship between the three variables, (1) species of fungus, (2) temperature, and (3) humidity, must be extremely complex. The results on *Aspergillus niger* will serve only to expose more fully the fundamental problem though their practical value will be more immediate.

SUMMARY

The combined temperature and relative humidity requirements of *Aspergillus niger* were studied with the following results: The optimum conditions for growth are at a relative humidity near 93 per cent and at a temperature near 40° C.; at 100 per cent relative humidity the optimum temperature is near 30° C. Thus for this species the optimum condition for growth is not at saturation and the optimum temperature changes with different humidities.

A general survey of the literature shows that the variability among fungi in their response to temperature and humidity is great, indicating that there is no simple temperature-humidity relationship for fungi.

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EXPLANATION OF FIGURES

FIG. 2. Graph showing the effect of temperature (ordinate) and relative humidity (abscissa) on the germination time (family of curves) of *Aspergillus niger*.

FIG. 3. Photomicrographs showing the effect of different combinations of temperature and humidity on the morphology of the germinating spore of *Aspergillus niger*. A. (300×) Spores germinating at high temperature (45° C.) and high relative humidity (100%). B. (300×) Spores germinating under optimal conditions (40° C., 93% RH). C, D. (300×) Spores germinating at low relative humidities (70-78%) and optimal temperatures (30-40° C.). E, F. (75×) Growth obtained if the fungus is kept for an extended time (3-5 weeks) under dry conditions (70-78% RH) at an optimal temperature (30° C.). Note how the hyphae spread out into fan-like processes and that in F normal sporangiophores can be seen.

TWO SPECIES OF COPRINUS WITH NOTES ON THEIR CULTURAL CHARACTERS

MORTEN LANGE¹

(WITH 4 FIGURES)

The present paper deals with two species of *Coprinus*; the one being identified with a previously described but little known species, the other believed to be previously undescribed. Both have been grown in culture and notes are given about their cultural characters. The descriptions, however, are drawn from material grown under natural conditions, if not otherwise stated.

COPRINUS COTHURNATUS Godey ap. Gillet 1878. (FIG. II, III)

Pileus 1.2–2.0 cm. high before expanding, ovate, subovate, expanding through campanulate to almost flat, slightly umbonate, edge recurving and splitting irregularly; whitish then grayish, plicate-striate nearly to the centre, disc not sharply delimited. Veil rather prominent on young specimens, of a dense, granulose to somewhat filamentous covering (especially towards the margin, which on young buds is connected with the stipe by fibrils), dingy whitish, in places with a reddish avellaneous tinge (conf. below), on mature specimens left as a few scurfy, brownish patches on top of cap. Flesh very thin, watery white. Lamellae free, narrow, rather crowded, with a varying number of smaller ones between; white, turning black over all (through pale pinkish cinnamon); spores ripening simultaneously, edge floccose, remaining white until deliquescing. Stipe 5–6 cm. long, 3–5 mm. thick, terete or slightly compressed, attenuated upwards, white, scabrose-tomentose from short fibrils (very much so towards the base which is also covered with distinct, granulose veil-remnants and tinged reddish avellaneous), distinctly hollow, not fragile. Odor and taste nauseating, somewhat like *Coprinus narcoticus*, rather pronounced. Sporeprint black.

¹ University of Copenhagen, Botanical Laboratory. The main part of this work was carried out while the author was studying in the laboratory of Dr. A. H. Smith, University of Michigan Herbarium, Ann Arbor, Michigan, United States of America.

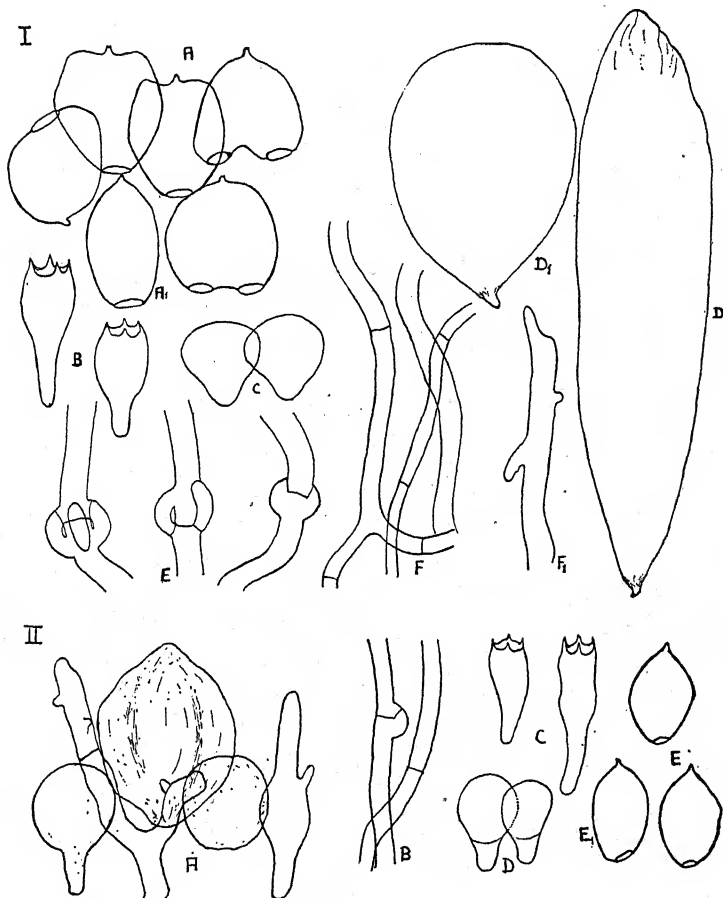
Spores $11.6-14.2 \times 7.6-9.5 \times 8.3-10 \mu$, mostly $12.5-13 \times 8-9 \mu$, broadly ovate, usually slightly flattened, some of them obscurely angular in face view, dark umber in H_2O (coal black in KOH), truncate, germ pore conspicuous, slightly eccentric, about 2.5μ broad. Basidia 4-spored, more or less dimorphic, $30-45 \times 10-11 \mu$, sterigmata $2-3 \mu$ long. Paraphyses pear-shaped. Pleurocystidia $18-30(50) \times 15-40 \mu$, scattered to rare, vesiculose and soon collapsing. (All material raised in culture has been absolutely devoid of pleurocystidia). Edge of gills made up of pear-shaped, subglobose or globose cells, $15-35 \mu$ broad, borne on thorn-like projections on cylindric filaments $3-8 \mu$ broad. Gill trama interwoven, cells about $3-5 \mu$ broad. Veil of cap similar to the tissue of the gill edge but inflated cells averaging somewhat larger ($15-80 \mu$), slightly incrustated, readily collapsing, often with brownish amorphous material between. Clamp connections demonstrated on mycelium (see below) but not in fruitbody.

Habit, habitat and distribution. Fasciculate, in large clusters, on old, decaying haystack. May 21-25, 1947, Arboretum, Ann Arbor, Mich., U. S. A., leg A. H. Smith and M. Lange (Lange 718, 719). Previously recorded from France, Switzerland and England.

Observations. The specimens grown *in vitro* show a very prominent veil, which on young buds forms conspicuous warts. Stem and veil both turn brick red a few seconds after being touched and after some minutes fade to vinaceous brown or avellaneous. These characters are more difficult to ascertain on specimens found in nature, but are very striking on those produced in culture.

The peculiar color reaction of the veil and stem gives *C. cothurnatus* some similarity to *C. dilectus* and *C. roseotinctus*, but I do not believe there is any close relationship since these species have another type of veil. *C. cothurnatus* evidently belongs in the group of *C. niveus* and *C. semilanatus* by virtue of similar veil and spore characters, and appears to be very close to the latter, from which the changing color of the veil and differences in shape and size of spore distinguish it. It is not without some doubt that I give this interpretation of *C. cothurnatus*, a species badly treated or ignored in modern literature, but the original description, as given by Saccardo, fits my plant well, except for the mention of the gills turning black through "flesh color." No detailed description of the microscopic characters of *C. cothurnatus* is known to me. Al-

though the plant originally was recorded from cow dung, Martin (1904-05) finds it on humus, in the spring, thus better corresponding—if correctly interpreted—to the present find. I have had no access to Gillet's work (l. c.) but have seen a copy of the illustra-



FIGS. I-II. Microscopic characters of *Coprinus cothurnatus* and *C. myceliocephalus*.

tion (kindly made for me by Dr. J. Favre in Geneva)—it very much recalled the present plant.

Cultural characters. Spores spread on horse dung agar germinate readily in less than 24 hours at 18° C. (almost 100%). Poly-

spored mycelium is provided with clamp connections. Single-spore mycelia were isolated and mated together (TAB. 1). The species, accordingly, is heterothallic and bipolar. Fruitbodies form rather readily about a month after the inoculation both on agar in test tubes and on sterilized horse dung.

TABLE 1

Coprinus cothurnatus GOD. RESULT OF PAIRING OF 10 HAPLOID MYCELIA.

+ indicates formation of clamp connections and spore producing fruitbodies.

	A	C	D	H	F	E	G	B	I	J
A		-	-	-	+	+	+	+	+	+
C	-		-	-	+	+	+	+	+	+
D	-	-		-	+	+	+	+	+	+
H	-	-	-		+	+	+	+	+	+
F	+	+	+	+		-	-	-	-	-
E	+	+	+	+	-		-	-	-	-
G	+	+	+	+	-	-		-	-	-
B	+	+	+	+	-	-	-		-	-

Coprinus myceliocephalus sp. nov. (FIG. I, IV)

Pileus 0.4-0.8 (2.0) cm. altus, initio globosus, postea ex ovato expansus, velo universali valido, albo, in squamas adpressas lacerato obtectus, sub velo striatus, ex pallide cinnamomeo nigricans. Lamellae liberae, subconfertae, ventricosae, initio albae, dein cinnamomeae, tandem nigrae, marginibus flocculosis, albis. Stipes 4×0.2 (12×0.3) cm., flocculosus, cavus, albus. Odor et sapor nulli. Sporae (12.5) $14-17 \times 10-12.5$ (14) $\times 9-9.5 \mu$, subcordatae, subangulatae. Pleurocystidia $140-175 \times 25-40 \mu$, cylindrica, saccata. Cheilocystidia $50-150 \times 25-40 \mu$. Basidia tetraspora. Velum universale ex hyphis mycelioideis, $2-8 \mu$ latis contextum. *Typus* (M. Lange C. 88) in Mus. Bot. Hauniensi et in Herb. Univ. Mich. depositum, e fimo vaccinum ex Brewster Co. Texas Americae borealis habito natum aluit M. Lange, 5-5-48.

Pileus 0.4-0.8 cm. high before expanding (in culture up to 2.0 cm. high), nearly globose at first, then ovate, expanding to \pm umbonate, with edge recurving and somewhat splitting at maturity. Veil prominent, a thick, white, fibrillose coating, breaking up in adpressed patches during expansion and still conspicuous on deliquescent cap, the larger patches especially on and around the umbo. Surface of cap exposed between patches when expanding, somewhat viscid, watery cinnamon-brown, turning black through grayish brown. Edge of cap fibrillose. Flesh whitish, thin. Lamellae free, moderately crowded, 2-3.5 mm. broad, white at first, soon pale grayish brown, then blackening through ashy gray. Edge prominently white floccose at first, deliquescing from the edge. Spores ripening simultaneously on whole gill. Stipe 4 cm. long, 2 mm. thick (in culture up to 12×0.3 cm.), white; young stipe densely clad by a white filamentous coating, when old with

more scattered fibrils; cylindric, slightly tapering above, the base slightly bulbous, with remnants of the velum partiale; hollow, flesh whitish. Odor and taste faint. Sporeprint black.

Spores $(12.5)14-17 \times 10-12.5(14) \times 9-9.5 \mu$, distinctly flattened, \pm angular-subcordate in face view, elliptic in side view, impellucid, nearly black (coal black in KOH); germ-pore large, apical or nearly so; some few spores deformed, with two germ-pores. Basidia 4-spored, somewhat dimorphic, broadly clavate, short to long pedicellate, $25-40 \times 12-14 \mu$ incl. sterigmata (about

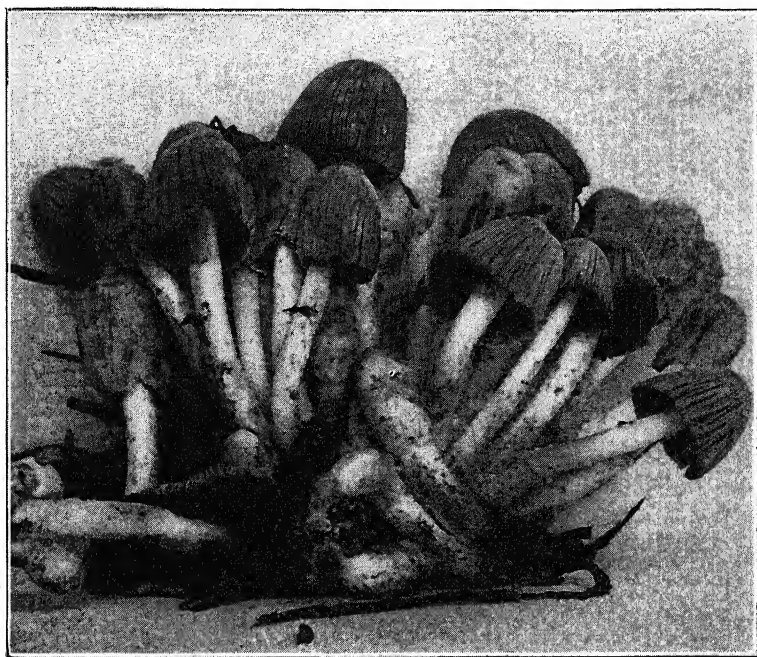


FIG. III. *Coprinus cothurnatus*.

4μ). Pleurocystidia rather numerous, $140-175 \times 25-40 \mu$, inflated cylindric, sac-shaped, apex broadly rounded to somewhat acuminate, thin-walled, readily collapsing. Cheilocystidia numerous, similar to pleurocystidia or considerably shorter, some being nearly globose, $50-150 \times 25-45 \mu$. Paraphyses broadly pear-shaped. Gill trama not prominent, interwoven, of elongated, somewhat branched cells, generally $5-10 \mu$ broad, some few inflated, much broader. (A section of young bud shows a loosely interwoven tissue between gill edges and stem, of which the cheilocystidia form a part.) Veil on cap made up of interwoven hyphae

of a mycelioid character, $2-8\ \mu$ broad. Outer layer of veil in young buds shows a considerable number of narrow but rather thick-walled hyphae. Nuclei are seen mostly in this part of the veil on fixed and stained material. On older caps the veil consists almost exclusively of somewhat wider, more thin-walled and irregularly branched hyphae. The filaments on the stem are made up of shorter hyphae of this latter type. No clamp connections seen in any part of fruitbody, but demonstrated in mycelium (conf. below).

Habit, habitat and distribution. Single, occasionally few together, on dung of cow and goat. Texas, Galapagos Islands.

Material studied. M. Lange C. 88-type, developed *in vitro* in laboratory on cow dung from SE. of Santiago Peak, Brewster Co., Texas, U.S.A. May 5, to 11, 1947 (the dung collected April 7, by R. McVaugh); M. Lange C. 36, Cultures obtained from goat dung collected on the Galapagos Islands were furnished through the courtesy of Professor G. W. Martin, Univ. of Iowa, Iowa City, Iowa.

Observations. The description is drawn from specimens which occurred with other Coprini on the moistened dung, and should be considered exactly corresponding to what will be met in nature. Of the Galapagos plant I have seen only specimens raised in pure culture, on sterilized substrate. These differ slightly in average size and shape of spores: $16-18 \times 12.5-14.5(15) \times 9.5-10\ \mu$, some of them more distinctly angular, and many of them (in some mounts up to 20 per cent) with two germ pores; these spores often collapse before maturity and very likely are sterile. I deem this character much too inferior to give the Galapagos plant the rank of a distinct species or variety.

The closest relative previously described in literature seems to be *C. vermiculifer* Joss. (Josserand, 1944). It has the same type of veil, especially in young stages, and the same type of gills with a floccose edge occasionally splitting. Microscopically the species are easily separated on account of the very different spores and the lack of clamp-connections in the veil tissue. Macroscopically the species must be very hard to distinguish. I have looked for adequate descriptions of similar species from the tropics and subtropics, but the generally very brief diagnoses have not permitted me to identify *C. myceliocephalus* with any of them.

CULTURAL CHARACTERS. The species fruits very readily on horse dung agar as well as on sterilized dung, sometimes as early as 11 days after the inoculation and after the development of a very vigorous white, aerial mycelium. The spores germinate in a very

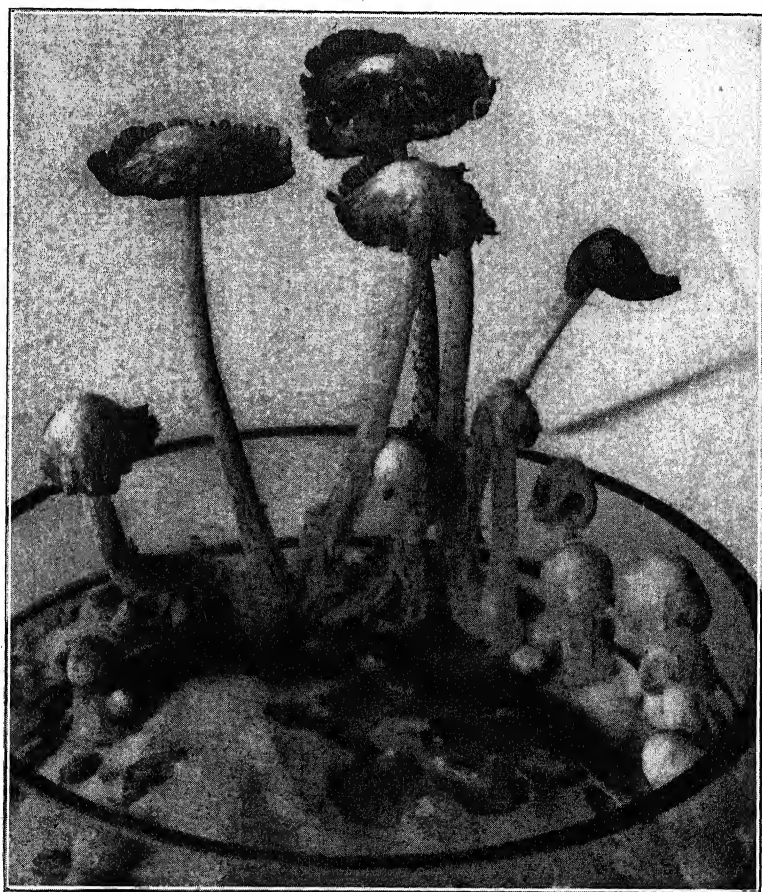


FIG. IV. *Coprinus myceliocephalus*.

low percentage on horse dung agar, generally only 0.01-1 per cent. Several different treatments, namely heating of the spores in juice of dung, addition to the agar of other types and concentrations of dung juice, and of various mineral nutrient, were tried with little or no effect. The germination of spores and development of one

mycelium on an agar-plate seem to stimulate the germination of the neighboring spores, and some young mycelia will generally develop in the outskirts of the older one. The first hyphae grow close to the agar surface, but in a distance of about 2-3 mm. from the germinating spore aerial hyphae develop, some of which are provided with very prominent and well developed clamp-connections, two or three at the same transversal wall (FIG. 1e). As far as I know, this phenomenon, described for instance from *Coniophora*, has not previously been noticed for a species of *Coprinus*, in spite of the thorough study of the cultural characters of this genus. It has been observed as a regular feature of all mycelia studied of this species including the single-spore mycelia, but the clamp-connections seem to occur with a varying frequency from plate to plate. They are most constantly seen on the first aerial hyphae, and never observed on hyphae growing down in the agar. Ten single-spore mycelia were isolated (from both strains). All produced normal fruitbodies, the spores of which gave rise to a second generation. From this it is evident that the species is truly homothallic.

Temperature: Mycelia were placed in constant temperature chambers at different temperatures. As one would expect, knowing the place of the origin of the cultures, the growth was very vigorous at 35° C., slightly slower at 22° C., and at 15° C. nearly zero. Cultures kept at 18° C. will sometimes develop normally, but sometimes stop growing after a while. The lower limit for normal growth thus appears to be about 20° C. and is considerably higher than for *Coprinini* from cooler climates.

Light: The species requires light for normal fruiting. In the dark, long stipes develop with small brownish caps, the veil becomes very much reduced, and these caps never open. They collapse after a while without ripening spores. Some cultures developed no buds at all in the dark.

ACKNOWLEDGMENTS. My work on the *Coprinini*, to which this paper is a first contribution, has been suggested by Dr. A. H. Smith, who most kindly placed a considerable amount of material at my disposal, and gave valuable advice. To the Director of the University of Michigan Herbarium, Dr. E. B. Mains, and also to the directors of the Carlsberg Laboratorium and the Botanical

Laboratorium of the University in Copenhagen, where my culture work was carried out, I owe a debt of thanks for granting me the necessary facilities. The latinizing of the diagnosis is by Tyge Christensen.

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EXPLANATION OF FIGURES

I. *Coprinus myceliocephalus*. A, spores in face view; A₁, spore in side view; B, two basidia; C, two paraphyses; D, pleurocystidia; D₁, short cheilocystidium; E, hyphae from mycelium with triple and double clamp-connections; F, hyphae from veil of young cap; F₁, hyphae from flocci on stem; (A and A₁ × 1000; B-F × 500.)

II. *Coprinus cothurnatus* God. A, elements from veil on cap; B, mycelium with clamp-connection; C, two basidia; D, two paraphyses; E, two spores in face view; E₁, spore in side view. (A-D × 500; E and E₁ × 1000.)

MYCOLOGICAL NOTES. IX

C. L. SHEAR

37. THE GENUS *VENTURIA* AND A PROPOSED LECTOTYPE

De Notaris in his original publication of the genus *Venturia* (Atti Sci. Ital. 6: 484. 1844) described two species, *V. rosae* and *V. dianthi*. It might naturally be expected that subsequent authors would have based their concept of the genus on one or the other of these species, but this was not the case, the type method of fixing the application of generic names having not yet been proposed. Cesati and de Notaris (Comment. Soc. Crittogam. Ital. 1: 225. [Reprint p. 51.] 1863) transferred other species to the genus, including *V. dickei* (Berk. & Br.), *V. eres* (Berk. & Br.), *V. macrotrichia* (Berk. & Br.), and *V. chaetomium* (Cda.).

Saccardo (Sylloge Fungorum 1: 586. 1882) took the genus in the sense of Cesati and de Notaris (l. c. 1863) "pro minore parte" rather than as first set up by de Notaris. He noted that the two original species were setose Pleosporas for which at a later date (Syll. Fung. 2: 285. 1883) he established the genus *Pyrenophora*. In his compilation he included species referred to the genus by Fries, Cooke, Karsten, and others as well as a number added by himself to make up a heterogeneous group of forty species.

In 1886 Berlese and Saccardo (Ann. Soc. Ven. Trent. 10: 174) proposed a new genus, *Proventuria*, based on the original *Venturia rosae* de N. (*Pyrenophora rosae* [de N.] Sacc.), a study of authentic specimens having shown that the ascospores were uniseptate and brown rather than muriform. The other original species, *V. dianthi*, was renamed *Pyrenophora notarisii* Sacc. (Syll. Fung. 2: 285. 1883) as already noted.

Since the publication of the Sylloge the general usage of mycologists and phytopathologists has been that of Saccardo. However, even a casual study of the various species included by Saccardo shows that several genera are represented, so that in order to apply the name in a definite manner and to avoid further confusion, the

name should be conserved and typified by a species representing the most general current usage. The best known group of species is that including *V. inaequalis* (Cke.) Wint. and *V. pyrina* Aderh. On account of their great economic importance as the cause of apple and pear scab they have been thoroughly investigated. Their conidial form is *Fusicladium*. Until we have more complete information in regard to many of the other species now included in the genus no satisfactory, complete segregation can be made.

Clements and Shear (The Genera of Fungi, p. 267. 1931) proposed as the type *V. chlorospora* (Ces.) Karst. which Ellis and Everhart (No. Amer. Pyren. p. 138. 1892) noted as a synonym of *V. inaequalis* (Cke.) Wint., although they are now regarded as distinct species. But it is perhaps better to take *V. inaequalis* as the type since it is the more familiar species. Bisby and Mason (Trans. Brit. Myc. Soc. 24: 172. 1940) state that *Venturia* should be conserved and included in their list of species, *V. aucupariae*, *V. chlorospora*, *V. inaequalis*, and *V. pirina*.

In 1923 Sydow (Ann. Myc. 21: 171) protested the action of Saccardo and Berlese, as mentioned above, in transferring the two original *Venturia* species of de Notaris to other genera and using the name for another group of heterogeneous species. He proposed a new genus, *Spilosticta*, typified by *V. rumicis* (Desm.) Cke. and added a new species, *S. bistortae*. Species of this genus were characterized by buried foliicolous perithecia and an *Ovularia* conidial stage. For the leaf inhabiting species with a *Fusicladium* conidial stage he proposed the genus *Endostigme* to include the following species placed in *Venturia* by Saccardo: *E. inaequalis* (Cke.) (type), *E. ditricha* (Fr.), *E. tremulae* (Aderh.), *E. chlorospora* (Ces.), *E. fraxini* (Aderh.), *E. pirina* (Aderh.), and *E. crataegi* (Aderh.). *Endostigme* would then be an exact synonym of *Venturia* as proposed for conservation and typified by Clements and Shear and Bisby and Mason.

Petrak (Ann. Myc. 38: 193. 1940) considered Sydow's two genera synonymous and made the new combination *Spilosticta inaequalis* (Cke.) Petr. for the apple scab fungus. Jørstad (Nyt. Mag. Nat. 84: 251-3. 1943) accepted Sydow's genera *Spilosticta* and *Endostigme* as distinct because of their different conidial stages. For the type species of the latter, however, he proposed to substi-

tute *Sphaerella cinerascens* Fleischhack for *E. inaequalis*, making the new combination *E. cinerascens* (Fleischhack) Jørstad, since he considered *cinerascens* the oldest valid specific name for the apple scab fungus. This action does not appear to be justified for the following reasons:

Jørstad's decision was based on Rabenhorst's use of the name in 1865 for a fungus issued as No. 845 of his exsiccati, the label reading as follows:

Rabenhorst, Fungi europaei.

845. SPHAERELLA CINERASCENS Fckl. Fung.

Rhenan. N. 824.

Paraphysibus nullis, ascis tubulosis octosporis, sporis uniseriatis, pulchre chlorinis, uniseptatis, 1/76 mm longis a *Sphaerella ditricha* Fr., et *Sphaerella chlorospora* Cesati vix diversa.

In foliis Sorbi ariae legit Arnstadiae (Thuringiae) Majo 1865.

Dr. Fleischhack.

There is nothing here to justify the conclusion that Fleischhack was the author of the description or the name, since Rabenhorst clearly cites Fuckel as the author of the species and Fleischhack as the collector. This error in ascribing the authorship of *Sphaerella cinerascens* to Fleischhack apparently originated with M. C. Cooke (Jour. Bot. 4: 248. 1866), who included it as a synonym in describing *Sphaerella inaequalis* as a new species common in England on dead leaves of ash, hawthorn, pear, and apple: "*Sphaerella cinerascens*, Fleisch. Rab. Fung. Eur. no. 845 (not *S. cinerascens*, Fuckel, Fung. Rhen. no. 824." This synonymy is accepted on the basis of Cooke's statement since we have been unable to find the fungus itself on the specimen of Rabenhorst, No 845, in the Mycological Collections of the Bureau of Plant Industry. It will be noted that the label on this specimen as cited above credits the binomial *Sphaerella cinerascens* to Fuckel on the basis of a specimen issued in the latter's Fungi Rhenani, No. 824. However, the Fuckel specimen was issued under the name *Sphaeria cinerascens* so that Rabenhorst's usage constitutes a new combination, although it was again cited by Fuckel in 1869 (Symb. Myc. 103) as *Sphaerella cinerascens* Fckl. Both Cooke (l. c.) and Fuckel (l. c.) state that *Sphaerella cinerascens* Fckl. in Rab. Fung. Eur. no. 845 and *Sphaerella* (*Sphaeria*) *cinerascens* Fckl. in Fung. Rhenani no.

824 are different fungi, but this is irrelevant to the present discussion.

After all the specific epithet *cinerascens* is invalid for the apple scab fungus since *Sphaeria cinerascens* Fckl. is a later homonym of *Sphaeria cinerascens* Schw. (Syn. Fung. Am. Bot. p. 225. 1832) described on leaves of *Asclepias*. Cooke transferred this species to *Sphaerella* (Jour. Bot. 21: 130. 1883) and Saccardo (Syll. Fung. 1: 31. 1883) considered it a *Laestadia*. With the elimination of *cinerascens*, *inaequalis*, the epithet most generally used in the extensive literature on the apple scab fungus, remains acceptable. Winter appears to have been the first to make the transfer from *Sphaerella* of Cooke to *Venturia* in 1875, or at least it is credited to him by de Thuemen on the label of his specimen No. 261, Mycotheca universalis. Aderhold (Hedwigia 36: 81-2. 1897) in his careful studies of a related group of *Venturia* species restricted the name *V. inaequalis* to the fungus on *Malus* and *Pyrus* (other than *P. communis*), drew up an emended description, and credited the combination to himself. A correct citation of the species would appear to be *Venturia inaequalis* (Cke.) Wint. emend. Aderh., as noted by Jørstad (l. c.), which for ordinary usage becomes

VENTURIA INAEQUALIS (Cke.) Wint. apud de Thuemen Myc. Univ. Exsic. no. 261. 1875.

Sphaeria cinerascens Fckl. Fungi Rhen. no. 824. 1863. (non *S. cinerascens* Schw. Syn. Fung. Am. Bot. 225. 1832.)

Sphaerella cinerascens Fckl. apud Rab. Fungi Eur. no. 845. 1865. (non *S. cinerascens* Cke. Jour. Bot. 21: 130. 1883.)

Sphaerella inaequalis Cke. Jour. Bot. 4: 248. 1866.

Venturia inaequalis Aderhold Hedwigia 36: 81-2. 1897.

Didymosphaeria inaequalis Niessl in Rabh. Fung. Eur. Exsic. no. 2663. 1881.

Endostigme inaequalis Syd. Ann. Myc. 21: 171. 1923.

Spilosticta inaequalis Petr. Ann. Myc. 38: 193. 1940.

Endostigme cinerascens Jørstad Nytt. Mag. Nat. 84: 252. 1944.

38. VENTURIA ON ERICACEAE

There is a group of closely related species usually referred to *Venturia*, which are found on leaves of ericaceous plants. They

differ from the type species, *V. inaequalis*, in having superficial perithecia and no known conidia. They appear to be nearer to *Coleroa* Rab. as typified by *C. chaetomium* (Kze.) Rab., which occurs on *Rubus*. Paraphyses are usually uncertain or wanting. *Gibbera*, as typified by *G. vaccinii* Fr., has *Helminthosporium* conidia and paraphyses and does not seem congeneric. The species to be considered are three.

Venturia arctostaphyli Cke. & Hark. Grev. 13: 20. 1884.

This species was originally described from dead leaves of *Arctostaphylos* collected in California. An examination of part of the type material collected by Harkness on leaves of *A. pumila* shows small superficial perithecia scattered over spots on dead leaves. The surface of the spots has the grayish-white, somewhat glistening appearance common to this group of species. They lose something of this characteristic after infected leaves have fallen and become faded. We find mature spores $15-18 \times 5 \mu$, somewhat larger than the measurements recorded in the description ($12-15 \times 5 \mu$). Setae around the summit of the perithecia are from $50-75 \mu$ long. This does not seem to be specifically distinct from the fungus found on *A. uva-ursi* in Massachusetts, although in the eastern form, the setae on the perithecia are only $40-45 \mu$ long and not so numerous. This slight difference, however, could scarcely be considered specific.

Venturia cassandrae Pk. Report N. Y. State Bot. 38: 104. 1885.

Peck noted that this species caused reddish-brown or brownish spots, sometimes with a grayish center and that the perithecia were minute, broad, black, with a few short, straight, diverging black setae above. He found the fungus on living leaves of *Cassandra calyculata* in New York State. He adds further: "the perithecia sometimes occur on the upper surface of the leaf, but oftener on the lower. They are so small that they are scarcely visible to the naked eye. Sometimes they emerge from beneath the scales of the leaf, and then they appear erumpent, although in reality they are superficial."

Venturia gaultheriae Ell. & Ev. Jour. Myc. 1: 153. 1885.

The description of this species as taken from Ellis and Everhart's North American Pyrenomycetes follows:

"On orbicular, dark brown, $\frac{1}{3}$ mm. spots, which are mostly of a lighter color (gray) in the center. Perithecia scattered, orbicular ($75\ \mu$), membranous and rather coarsely cellular, with a few black, continuous, straight, spreading, $35 \times 3\ \mu$ bristles above. Asci ovate-oblong, $30-35 \times 8-11\ \mu$, broader and slightly curved below, sessile, without paraphyses. Sporidia biserial, subhyaline (with a greenish-yellow tint), ovate-oblong, 3-4-nucleate, uniseptate and slightly constricted at the septum, $11-14 \times 3\ \mu$.

"On living leaves of *Gaultheria procumbens*, Newfield, N. J."

From examination and comparison of type material and other authentic specimens we conclude that the three species described above are the same for which the oldest name *V. arctostaphyli* Cke. & Hark. may be used. We have found this species also on *Vaccinium macrocarpon*, the cranberry. Other species closely related and apparently congeneric are *V. pulchella* Cke. & Pk. and *V. kalmiae* Pk., differing from *V. arctostaphyli* in the size of the ascospores, which are $7-10 \times 3\ \mu$. Further study of these and other species such as *V. dickei* (Berk. & Br.) Ces. & de N. and *V. myrtilli* Cke. must be made in order to determine whether they deserve generic segregation.

39. VENTURIA CININNATA (Fr.) Fr.

Fries (Syst. Myc. 2: 451. 1823) described *Sphaeria cincinnata*, occurring very rarely on leaves of *Vaccinium oxycocum*. He later (Summa Veg. Scand. p. 405. 1849) transferred the species to *Venturia*.

Schweinitz (Syn. Fung. Am. Bor. p. 22. 1832) reported the species as occurring on leaves of *Oxycoccus macrocarpus* at Pocono, Pennsylvania. An examination of his specimen, however, indicates that it is not Fries' species, but what was later named by Peck (Rept. N. Y. State Bot. 25: 106. 1873) as *Venturia compacta* and transferred erroneously by the writer (U. S. Dept. Agric. Tech. Bull. 258: 13. 1931) to *Gibbera* which has a conidial stage. The above species has none.

Just what species Fries actually had is uncertain. We were unable to find any specimen under this name in his herbarium. Later reports of the species appear to be limited to three: Ellis and Everhart (North Amer. Pyren. p. 142. 1892) record it from Greenland on *Vaccinium palustre*; Rostrup (Bot. Tids. 27: 35 R. 1906) also reports it from Greenland and on the same host; and there is a specimen in the Mycological Collections of the Bureau of Plant Industry from Bresadola. This is labelled *Venturia cinnata* (Fr.) Rost. in his own handwriting and with no other data than the statement "Pyrenomycète sur les feuilles d' *Oxycoccus palustris* avec un croquis." The specimen is presumably of European origin. It consists of a number of dead leaves bearing on their lower surfaces perithecia typical of *Acanthorhynchus vaccinii* Shear. The sketch referred to on the label shows the typical short beak of this species with the stiff setae as well as the asci, ascospores and paraphyses characteristic of the species. Measurements given for the ascospores are $30-40 \times 12-15 \mu$. This is clearly not the fungus described by Fries who states that the perithecia of his fungus are entirely superficial, small, subparabolic and sometimes surrounded by curly hairs at the base. In the Bresadola specimen the perithecia are large, subglobose, and completely embedded in the leaf tissue.

No specimens have been seen on *Oxycoccus* or *Vaccinium* which agree with Fries' description. His fungus may be related to the *Venturia arctostaphyli* group, although we have never seen any specimens of this group with perithecia having curly basal hairs nor with conical-cylindrical perithecia. It may really be "sui generis" as Fries says.

40. NOTES ON ANTENNARIA, ANTENNULARIA, AND NIESSLIA

Antennaria as described by Link (In Schrader, Neu. Jour. Bot. 3: 16, 1809, not Gaert., 1791) was typified by *A. ericophila*. Link described and illustrated only the conidial form of the fungus. Nearly a century later Neger (Centralbl. Bakt. Par. 20 (2): 94. 1907) collected Link's fungus, which originally came from Portugal, in Andalusia on *Erica arborea*. He found perithecia as well as the typical *Antennaria* form of conidiophores described

and illustrated by Link. At an altitude of 200 to 400 meters there was but small growth of subiculum on the smallest branches of the host plant, and especially on the leaf axils and nerves of fallen leaves. This subiculum was small (about the size of a pin-head) and bore perithecia particularly in the leaf axils. The asci were $40-50 \times 15-20 \mu$, united in a mass which is typical of some *Venturia* species; the ascospores were 2-celled, greenish, and $18-20 \times 7 \mu$. At high altitudes, the mycelium of the fungus developed around the axes of the whole plant in dense black mycelial balls, in some instances reaching the size of one's fist. These were easily removed. They absorbed water like a sponge. At about 700 meters these masses developed a peculiar growth of erect *Antennaria* conidiophores 1-2 mm. high, bearing a head of 4-celled dark conidia. The column (saule) of conidiophores was composed of necklace-like hyphae and the interior hyphae bore club-shaped brown conidia, $40-50 \mu$ long. No perithecia were found in these mycelial balls. This, he thinks, was probably due to the greater humidity and rainfall, as well as the colder climate at this altitude. The fungus was not parasitic, he says, but its exclusion of the light and air from the host frequently weakened and killed the plants.

Von Hoehnel (Frag. Myk. no. 379. 1909) discusses *Antennaria* Link and states that nothing certain was known about its identity and relationships until Neger described it as discussed above. After an examination of Neger's specimens von Hoehnel said that he was convinced *Antennaria ericophila* Lk. was identical with *Coleroa straussii* (Sacc. & Roum.) Hoehn. (*Venturia straussii* Sacc. & Roum.), but that no *Antennaria* form of *C. straussii* was known. Further investigation, he said, showed that when mature the perithecia had a distinct ostiole and that conidia were found.

Venturia straussii Sacc. & Roum. (Rev. Myc. 6: 95. 1884) was based on material collected in 1884 by M. Merlet on *Erica scoparia* near Bordeaux, France, and sent by him to Roumeguère. Saccardo, who received the specimen for study, considered it identical with the fungus described and illustrated by Strauss (in Sturm, Deutschland Flora, Abt. III, Heft 34: 29-30, Tab. 3, Figs. A-G. 1853) under the name *Chaetomium pusillum* Fr. Strauss' identification, however, was in error, as was indicated by Saccardo's action in setting up a new name for the Merlet specimen.

The original Friesian fungus was referred to *Niesslia* by Schroeter and also named de novo by Albertini and Schweinitz and Corda as the following partial synonymy will show. This species occurring on pine needles should not be confused with the two species on Ericaceous hosts discussed hereafter.

NISSLIA FUSILLA (Fr.) Schroet., in Cohn, Krypt. Fl. Schl. 3: 294. 1893.

Sphaeria exilis Alb. & Schw. Conspect. Fung. 44. 1805.

Chaetomium pusillum Fr. Syst. Myc. 3: 255. 1829.

Sphaeria chaetomium Corda Icones II, 29. 1838.

Venturia chaetomium Ces. & de N. Schema Class. Sfer. Ital. 225. 1863.

Niesslia chaetomium Auers. in Gonnerman Rab. Mycol. Eur. Heft V & VI 30. 1869.

Niesslia exilis Wint. in Rab. Krypt.-Fl. 2 Aufl., 1 Bd., II Abt. 196. 1885.

The fungus described and illustrated by Strauss (l. c.) was found on living leaves of *Erica carnea* near Munich. No type material is known. There is little doubt, however, that *Venturia straussii* Sacc. and Roum. is the same and that as von Hoehnel states it is the same as *Antennaria ericophila* (Lk.) Neger.

The name *Antennaria* Link being untenable because of an earlier use of the name for a phanerogamic plant by Gaertner (1791), Reichenbach in 1828 (Consp. Reg. Veg. p. 5) proposed *Antennularia* as a substitute. This was also invalid for an ascogenous fungus until the perithecial form was found and described by Neger and adopted by von Hoehnel as discussed above. We regard the genus *Antennularia* (Reich.) Hoehn. as having been validated by von Hoehnel (Frag. Myk. 379. 1909) when he described Neger's specimens of *Antennaria ericophila* Lk., which showed conidia, perithecia, and ascospores, and adopted Reichenbach's name for the fungus.

He added three other species to the genus in addition to the type, *A. ericophila* (Lk.) Hoehn., among them being *A. salisburgensis* (Niessl) Hoehn. The original material upon which the description of this latter species was based was distributed in 1886 as no. 3550 in Rabenhorst—Winter's Fungi Europaei Exsiccati. After comparing specimens of this number with the original material of *Venturia straussii* Sacc. & Roum. (*Antennularia ericophila* (Lk.)

Hoehn.) issued by Roumeguère as no. 2828 of his Fungi Gallici Exsiccati and other specimens of the same collection issued as no. 3142 of Rabenhorst—Winter's Fungi Europaei, we are of the opinion that the two are different species. Von Hoehnel (Frag. Myk. no. 115. 1907) came to the same conclusion after studying specimens of the same numbers. The chief difference is that in *A. ericophila* the perithecia form dense masses in the axils of the leaves and on the stems whereas in the second species they appear to be confined to the leaves. The ascospores in the first are shorter and broader ($15-18 \times 6-8 \mu$) than in the second ($18-21 \times 5-6 \mu$).

Von Hoehnel has added two other species to this genus, *A. engleriana* (P. Henn.) Hoehn. (Frag. Myk. no. 356. 1909) based on *Dimerosporiopsis englerianus* P. Henn. and *A. rhododendri* Hoehn. based on the ascogenous form of *Torula rhododendri* Kunze. We have seen no authentic specimens of either of these and therefore can express no opinion regarding them. The synonymy of the two species which we have studied is as follows:

ANTENNULARIA ERICOPHILA (Neger) Hoehn. Sitzungsab. Akad. Wiss. Wien. 118, abt. 1: 1198. 1909.

Antennaria ericophila Lk. in Schrader Neues Jour. Bot. 3: 16. 1809.

Venturia straussii Sacc. & Roum. Rev. Myc. 6: 95. 1884.

Antennaria ericophila Neger, Centralbl. Bakt. 20 (2): 94. 1897.

Coleroa straussii Hoehn. Sitzungsab. Akad. Wiss. Wien 116, Abt. 1: 115. 1907.

Exsiccati specimens examined: Roumeguère, Fungi Gallici exs., no. 2828, as *Venturia straussii* Sacc. & Roum.; Rabenhorst—Winter Fungi Europaei, no. 3142, as *V. Straussii* Sacc. & Roum.

ANTENNULARIA SALISBURGENSIS (Niesel) Hoehn. Oesterr. Bot. Zeit. 63: 233. 1913.

Chaetomium pusillum Strauss in Sturm, Deutschland Flora, Abt. III, Heft 34: 29-30. 1853, non Fr. 1829.

Gibbera salisburgensis Niessl Hedw. 26: 33. 1887.

Eriosphaeria salisburgensis Neger Ber. Deutsch. Bot. Gesell. 19: 471. 1901.

Coleroa salisburgensis Hoehn. Sitzungsab. Akad. Wiss. Wien 116, Abt. 1: 115. 1907.

Gibbera straussii Zahlb. Krypt. Exs. Mus. Pal. Vind., no. 824.

Exsiccati specimens examined: Jaap, Fungi Sel. Exs., no. 614, as *Antennaria salisburgensis* (Niessl) Hoehn.; Zahlbruckner, Krypt. Exs. Mus. Pal. Vind., no. 824, as *Gibbera straussii* Zahlb.; Rabenhorst-Winter, Fungi Europ. Exs., no. 3550, as *Gibbera salisburgensis* (Niessl) Hoehn.; Rehm, Ascom. Exs., no. 1939, as *Coleroa salisburgensis* (Niessl) Hoehn.

The fungus issued by Jaap (Fungi Sel. Exsic. no. 657) as *Antennaria salisburgensis* (Niessl) Hoehn. associated with the aphid, *Eriococcus ericae*, on *Erica tetralix* is doubtfully the same as his no. 614. It has perithecia with the short, stiff setae and the colored ascospores of the same size and shape as the other specimen, but the perithecia occur on the stems as well as on the leaves and show more of the long basal hyphae, which seem to have grown over some of the aphids. It will be necessary to learn more in regard to the relation between the aphids and this and related species of these fungi, especially *Antennularia ericophila*, before a final decision is possible.

A further specimen found in the Mycological Collections of the Bureau of Plant Industry should be considered here. It was issued by C. Torrend as no. 146 of his Fungi Selecti Exsiccati and is labelled "*Gibbera salisburgensis* Niessl, ad folia *Ericae arboreae*, Madère, C. de Menzes, VI-1912." It consists of a few leaves bearing on the under side small dense groups of perithecia without subiculum, appendages, or setae. The perithecia are slightly rough or minutely verrucose. The perithecial wall is much thicker than in the other species mentioned, but paraphyses, asci, and ascospores are typical. The spores are $15-20 \times 6-8 \mu$. This is not Niessl's species. It would seem to indicate that there may be another group of species without subiculum or setae, but otherwise alike.

Neger (Ber. Deutsch. Bot. Gesell. 19: 471. 1901) after examining the Rabenhorst-Winter specimen (no. 3550) of *Gibbera salisburgensis* collected the fungus himself on *Erica carnea*. His material agreed exactly with the other. He described it in detail and showed a leaf section which indicated that a dense mass of vegetative hyphae developed beneath the thin epidermis of the upper leaf surface from which the superficial subiculum and perithecia arose. Infection took place in the summer and perithecia formed the following year. He maintained that the fungus was not a

Gibbera and transferred it to *Eriosphaeria*, as *E. salisburgensis* (Niessl) Neger.

There has just come to our attention Petrak's paper "Über *Gibbera* Fr. und verwandte Gattungen" (Sydowia 1: 169-201. 1947) in which he revises the genus *Gibbera* with *G. vaccinii* Fr. as the type and includes *Antennularia* as a subgenus. He does not include the production of conidia as a generic character. We regard this as a character of sufficient importance to exclude such species as *Venturia compacta* and other superficial species having no conidia.

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MYCOLOGIA

FINANCIAL STATEMENT

(July 1, 1947-June 30, 1948)

Unexpended reserve, July 1, 1947	\$ 4,924.05
<i>Current receipts (joint funds):</i>	
Mycological Society (members' subscriptions) ..	\$1,864.00
Subscriptions	3,477.80
Sale of back sets (vol. 25 and later)	824.35
Payments for excess paging	388.52
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<i>Special funds:</i>	
Sale of back sets (vols. 1-24) and index	415.80
Interest on endowment	583.00
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	\$ 998.80
Total receipts	\$ 7,553.47
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Total on hand	\$12,477.52
<i>Cost of printing and distribution:</i>	
Printing, binding, mailing 6 issues	\$4,787.44
Engraving	1,209.73
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	\$5,997.17
Replacing exhausted issues	524.82
Miscellaneous office expenses	274.84
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Total cost	\$ 6,796.83
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Balance	\$ 5,680.69
Transferred to endowment fund	\$ 1,000.00
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Unexpended reserve, June 30, 1948	\$ 4,680.69
Endowment fund	14,000.00
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Total on hand	\$18,680.69

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